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(71) Applicants: **FRED HUTCHINSON CANCER RESEARCH CENTER** [US/US]; 1100 Fairview Avenue North, Seattle, Washington 98109 (US). **UNIVERSITY OF WASHINGTON** [US/US]; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US).

(72) Inventors: **TAYLOR, Justin J.**; 1100 Fairview Avenue North, Seattle, Washington 98109 (US). **SHEHATA, Laila**; 4545 Roosevelt Way NE, Suite 400, Seattle, Washington 98105 (US). **TOOLEY, Marti**; 1100 Fairview Avenue North, Seattle, Washington 98109 (US).

(74) Agent: **WINGER, C. Rachal**; Lee & Hayes PC, 601 W. Riverside Avenue, Suite 1400, Spokane, Washington 99201 (US).

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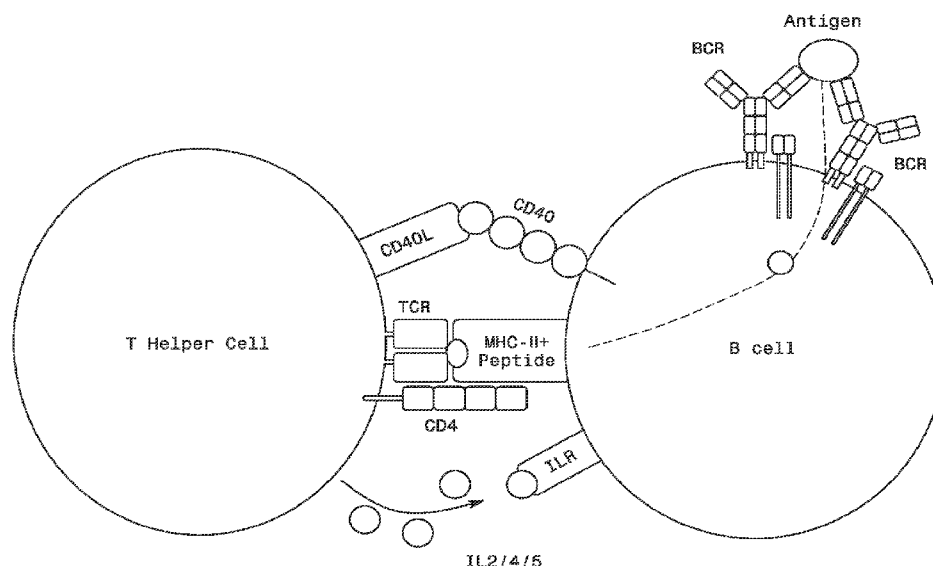


FIG. 1

(57) Abstract: Systems and methods to link CD40 signaling to antigen binding, independently of CD40 ligand binding are described. The systems and methods include fusion proteins including an extracellular antigen binding domain linked to an intracellular CD40 signaling domain.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

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- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
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- *with sequence listing part of description (Rule 5.2(a))*

SYSTEMS AND METHODS TO LINK CD40 SIGNALING TO ANTIGEN BINDING

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/147,041 filed on February 8, 2021, which is incorporated herein by reference in its entirety as if fully set forth herein.

REFERENCE TO SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is F053-0124PCT_ST25.txt. The text file is 202 KB, was created on February 8, 2022, and is being submitted electronically via EFS-Web.

FIELD OF THE DISCLOSURE

[0003] The present disclosure describes systems and methods to link CD40 signaling to antigen binding, independently of interactions with CD40 ligand. The systems and methods include fusion proteins that, when expressed by a cell, include an extracellular antigen binding domain linked to an intracellular CD40 signaling domain.

BACKGROUND OF THE DISCLOSURE

[0004] Vaccines are designed to increase the immunity of a subject against a particular infection by stimulating B cells to produce antibodies against the targeted infectious agent. Routine pediatric vaccination is a long-established clinical intervention with comparatively low risk and high efficacy. Unfortunately, however, vaccinations are not available for all infectious agents. As one example, every year in the United States, millions of children visit a doctor or emergency room due to infections with Respiratory Syncytial Virus (RSV).

[0005] In addition to combating infections, antibodies can also be useful as treatments for other conditions such as autoimmune diseases, and cancer. However, these antibody-based therapies typically require repeated injections of the antibodies to maintain efficacy.

[0006] Technologies have been developed to genetically modify B cells to express selected antibodies that bind targeted antigens (see WO2019/079772). Potent B cell responses, however, typically require CD40 expressed on the surface of B cells to interact with CD40 ligand (CD40L) expressed on the surface of CD4⁺ T cells. If T cells have not encountered the targeted antigen, such interactions may not sufficiently occur. Absent the required T cell interactions, B cells expressing antibodies may fail to adequately expand, survive, and differentiate.

[0007] B cell's natural dependence on T cell interactions also hampers the development of universal donor B cells as broadly applicable therapeutics. This is because T cells interact with B cells by binding to peptides presented on the B cell surface within major histocompatibility complex (MHC) class II molecules. Within the human population there are hundreds of different MHC alleles, with the most frequent allele only being present in 10% of the population. Unfortunately, the host immune system will reject any cell that expresses a different MHC allele than the host.

SUMMARY OF THE DISCLOSURE

[0008] The current disclosure describes B cells that receive CD40 activation signals independently of CD40 ligand (CD40L) binding. This advance is achieved by genetically modifying B cells to express fusion proteins that link extracellular antigen binding domains to CD40 signaling domains. In this manner, CD40 activation signals are triggered by antigen binding, independently of interactions with CD40L expressed by T cells.

[0009] The current disclosure provides two main approaches to link CD40 signaling to antigen binding. The first includes linking CD40 signaling domains to engineered extracellular antigen binding domains, such as scFv. In this approach, CD40 signaling is triggered when antigen binds the engineered antigen binding domain.

[0010] The second approach includes linking CD40 signaling to B cell receptor (BCR) signaling. When bound by antigen, BCR naturally transmit B cell activation signals through the intracellular proteins CD79 α and CD79 β . The current disclosure provides linking an intracellular CD40 signaling domain to CD79 α and/or CD79 β , such that when CD79 α and CD79 β signaling is triggered by antigen binding, CD40 signaling is also triggered.

[0011] When a subject donates his or her own B cells for genetic engineering (or is otherwise immunologically matched to a donor), no modifications regarding the B cell's MHC alleles are required. However, another advance described herein is deleting MHC class II molecules from the genetically-engineered B cells so that the B cells can be used as universal donor cells for all individuals. MHC class II molecule expression can be deleted by eliminating activity of one or more transcription factors required for MHC class II molecule expression. Such transcription factors include CIITA, TRAC, TRBC, B2M, RFX5, and RFXAP.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Some of the drawings submitted herein may be better understood in color. Applicant considers the color versions of the drawings as part of the original submission and reserves the right to present color images of the drawings in later proceedings.

[0013] FIG. 1. Schematic of T cell dependent B cell activation, showing a T cell (left), a B cell (right) and engagement of several interaction molecules. Adapted from Janeway et al, *Immunologie* (Berlin, 2002)

[0014] FIG. 2. The CD40-CD40L complex showing the trimeric form of CD40L wedged between two monomers of CD40. Adapted from An et al., "Crystallographic and Mutational Analysis of the CD40-CD154 Complex and Its Implications for Receptor Activation", J. Biol. Chem. 2011 Apr. 1; 286(13): 11226-11235 and PDB Accession 3QD6.

[0015] FIG. 3. A mapping of protein interaction with CD40 intracellularly. The grey boxes underlining the CD40 sequence (SEQ ID NO: 203) from left to right include the transmembrane region (up to residue 215), box-1 binding site (residues 222-229), TRAF binding sites (residues 231-238 and 250-254), and box-2 binding site (residues 260-270). Adapted from An et al., J. Biol. Chem. 2011 Apr. 1; 286(13): 11226-11235

[0016] FIG. 4. Schematic depicting B cell activities following activation.

[0017] FIG. 5. Schematics of exemplary fusion proteins disclosed herein. These depicted constructs each include an engineered extracellular binding domain (scFv), an extracellular tag (Strep), an extracellular spacer (CD40 or IgG segment), a transmembrane domain (CD40 or IgG segment), a CD40 intracellular signaling domain, and an intracellular tag (HA). Construct (+) additionally includes an FKBP12 dimerization sequence.

[0018] FIGs. 6A-6D. (6A) Schematic of a natural BCR complex depicting an extracellular antigen binding domain and CD79 α/β BCR signaling domains. (6B-6D) Schematics of BCR-CD40 constructs disclosed herein. (6B) Intracellular CD40 signaling domain linked to CD79 α and CD79 β subunits. (6C) Intracellular CD40 signaling domain linked to CD79 α . (6D) Intracellular CD40 signaling domain linked to CD79 β .

[0019] FIG. 7. Human B cells were purified from PBMC and stimulated for 2 days in vitro in the presence of CD40L, IL-2, IL-10, IL-15 and CpG prior to electroporation with a plasmid encoding construct #1. Cells were placed back in culture with media containing CD40L, IL-2, IL-10, IL-15 and CpG for an additional 24 hours. The media was then removed and replaced with media containing insulin and transferrin with or without 15 ug/mL tetramerized HIV Env (SF162) and incubated for an additional 24 hours. Cells were harvested and stained intracellularly for phosphorylated-p38 (p-p38), phospho-STAT3 (p-STAT3), and the HA tag expressed by the construct. The % of P-p38+ and P-STAT+ cells that expressed the construct (HA+, five-point star) or did not express the construct (HA-, triangle) are displayed. Bottom Panel: As a positive control for CD40 signaling, control cells were incubated in the presence (pentagon) or absence of CD40L (triangle) throughout the culture period.

[0020] FIG. 8. RAMOS B cells were transfected with constructs encoding mCherry and wild-type CD79 α , wild-type CD79 β , CD79 α with the CD40 signaling domain (CD79 β /CD40), and/or CD79 β with the CD40 signaling domain (CD79 β /CD40) prior to incubation with 5 ug/mL anti-human Ig for 48 hours. After incubation, cells were permeabilized and stained for phosphorylated-p38 and phosphorylated-STAT3. Cells that were + and – for the constructs were determined based upon the presence or absence of mCherry.

[0021] FIG. 9. RAMOS B cells were electroporated with gRNA targeting B2M and/or CIITA 48 hours prior to the analysis of MHCI and MHCII expression using flow cytometry. sgRNA sequences for CIITA (SEQ ID NO: 1) and B2M (SEQ ID NO: 2) are provided.

[0022] FIGs. 10A-10H. Sequence schematics in which coding sequence is aligned with amino acid sequence.

(10A) Schematic of Construct 1 (2520 bp). The coding sequence encodes the amino acid sequence including a human CD40 signal peptide - anti-gp120 scFV - a StrepTagII® - Gly linker - CD40 full protein - Gly linker -HA tag - P2A skipping element - mCherry reporter.

(10B) Schematic of Construct 2 (2085 bp). The coding sequence encodes the amino acid sequence including human CD40 signal peptide - anti-gp120 scFv - StrepTagII® - Gly linker - IgG hinge - Gly linker - CD40 transmembrane domain and CD40 intracellular domain - Gly linker - HA tag - P2A skipping element - mCherry reporter.

(10C) Schematic of Construct 3 (2082 bp). The coding sequence encodes the amino acid sequence including human CD40 signal peptide - anti-gp120 scFv - StrepTagII® - Gly linker; IgG hinge - Gly linker - IgG2 transmembrane domain; Gly linker - CD40 intracellular domain - Gly linker - HA tag - P2A skipping element - mCherry reporter.

(10D) Schematic of Construct 4 (3225 bp). The coding sequence encodes the amino acid sequence including human CD40 signal peptide - anti-gp120 scFv - StrepTagII® - Gly linker - full CD40 protein - Gly linker - FkBP12v36 - GlySer linker- FkBP12v36 - Gly linker - HA tag - P2A skipping element - mCherry reporter.

(10E) Schematic of Construct 5 (1692 bp). The coding sequence encodes the amino acid sequence including human CD40 signal peptide - full CD40 protein - Gly linker - HA tag - P2A skipping element - mCherry Reporter.

(10F) Schematic of MRT3_PTT3_mouse CD79 α _IntracellularCD40 (7736 bp) in which CD40 is linked to mouse CD70 α . The coding sequence encodes the aligned amino acid sequence including mouse CD79 α - intracellular CD40 - P2A skipping element -T2A skipping element - mCherry reporter.

(10G) Schematic of MRT4_PTT3_mouse CD79 β _Intracellular CD40 (7760 bp) in which

CD40 is linked to mouse CD79 β . The coding sequence encodes the aligned amino acid sequence including mouse CD79 β - intracellular CD40 - P2A skipping element - T2A skipping element - mCherry reporter.

(10H) Schematic of MRT5_Ptt3_humanCD79 α _Intracellular CD40 (7638 bp) in which CD40 is linked to human CD79 α . The coding sequence encodes the aligned amino acid sequence including human CD79 α - intracellular CD40 - P2A skipping element - mCherry reporter.

[0023] FIG. 11. Additional sequences supporting the disclosure. Sequences include:

coding sequence of Construct 1 (SEQ ID NO: 3) and protein sequence of Construct 1 including a human CD40 signal peptide (underlined), anti-gp120 scFv, a StrepTagII® (**bold**), Gly linker (double underlined), the CD40 full protein (**bold and underlined**), a Gly linker (double underlined), HA tag (*italicized*), P2A skipping element (**bold and italicized**), and an mCherry reporter (*italicized and underlined*) (SEQ ID NO: 4);

coding sequence of Construct 2 (SEQ ID NO: 5) and protein sequence of Construct 2 including human CD40 signal peptide (underlined), anti-gp120 scFv, StrepTagII® (**bold**), Gly linker (double underlined), IgG hinge (**bold, underlined, and italicized**), Gly linker (double underlined), CD40 transmembrane domain and CD40 intracellular domain (**bold and underlined**), Gly linker (double underlined), HA tag (*italicized*), P2A skipping element (**bold and italicized**), and an mCherry reporter (underlined and italicized) (SEQ ID NO: 6);

coding sequence of Construct 3 (SEQ ID NO: 7) and protein sequence of Construct 3 including human CD40 signal peptide (underlined); anti-gp120 scFv, StrepTagII® (**bold**), Gly linker, IgG hinge (**bold, underlined, and italicized**), Gly linker (double underlined), IgG2 transmembrane domain, Gly linker (double underlined), CD40 intracellular domain (**bold and underlined**), Gly linker (double underlined), HA tag (*italicized*), P2A skipping element (**bold and italicized**), and an mCherry reporter (underlined and italicized) (SEQ ID NO: 8);

coding sequence of Construct 4 (SEQ ID NO: 9) and sequence of Construct 4 including human CD40 signal peptide (underlined), anti-gp120 scFv, StrepTagII® (**bold**), Gly linker (double underlined), full CD40 protein (**bold and underlined**), Gly linker (double underlined), FkBP12v36 (**bold, underlined, and italicized**), GlySer linker, FkBP12v36 (**bold, underlined, and italicized**), Gly linker (double underlined), HA tag (*italicized*), P2A skipping element (**bold and italicized**), and mCherry reporter (underlined and italicized) (SEQ ID NO: 10);

coding sequence of Construct 5 (SEQ ID NO: 11) and protein sequence of Construct 5 including human CD40 signal peptide (underlined), full CD40 protein (**bold and underlined**), Gly linker (double underlined), HA tag (*italicized*), P2A skipping element (**bold and italicized**), and an mCherry Reporter (*italicized and underlined*) (SEQ ID NO: 12);

coding sequence of MRT3 (SEQ ID NO: 13) and protein sequence of MRT3 including mouse CD79 α (underlined), mouse intracellular CD40 (italicized), P2A skipping element (double underlined), T2A skipping element (bold), and an eGFP reporter (SEQ ID NO: 14);

coding sequence of MRT4 (SEQ ID NO: 15) and protein sequence of MRT4 including mouse CD79 β (underlined), mouse intracellular CD40 (italicized), P2A skipping element (double underlined), T2A skipping element (bold), and an eGFP reporter (SEQ ID NO: 16);

coding sequence of MRT5 (SEQ ID NO: 17) and protein sequence of MRT5 including human CD79 α (underlined), human intracellular CD40 (italicized), P2A skipping element (double underlined), and an mCherry reporter (SEQ ID NO: 18);

coding sequence of MRT6 (SEQ ID NO: 19) and protein sequence of MRT6 including human CD79 β (underlined), human intracellular CD40 (italicized), P2A skipping element (double underlined), and an mCherry reporter (SEQ ID NO: 20);

Anti-gp120 scFv - Linker - StrepTagII - Gly6 Linker- full CD40 protein - Gly6 Linker - HA tag (SEQ ID NO: 21);

Anti-gp120 scFv - Linker - StrepTagII- Gly6 Linker- IgG Hinge- Gly6 Linker- CD40 Transmembrane Domain - CD40 Intracellular Domain - Gly6 Linker - HA tag (SEQ ID NO: 22);

Anti-gp120 scFv - Linker - StrepTagII- Gly6 Linker- IgG Hinge- Gly6 Linker- IgG2 Transmembrane Domain - Gly6 Linker - CD40 Intracellular Domain - Gly6 Linker - HA tag (SEQ ID NO: 23);

Anti-gp120 scFv - Linker - StrepTagII - Gly6 Linker- full CD40 protein - Gly6 Linker- FkBP12v36 - GlySer Linker - FkBP12v36- Gly6 Linker - HA tag (SEQ ID NO: 24);

MouseCD79 α -IntracellularCD40 (SEQ ID NO: 25);

MouseCD79 β -IntracellularCD40 (SEQ ID NO: 26);

HumanCD79 α -IntracellularCD40 (SEQ ID NO: 27);

HumanCD79 β -IntracellularCD40 (SEQ ID NO: 28); and

human CD40 signal peptide (SEQ ID NO: 29); anti-gp120 scFv (SEQ ID NO: 30); full CD40 protein (SEQ ID NO: 31); CD40 extracellular domain (SEQ ID NO: 32); CD40 transmembrane domain (SEQ ID NO: 33); CD40 intracellular domain (SEQ ID NO: 34); human intracellular CD40 domain (SEQ ID NO: 35); murine intracellular CD40 domain (SEQ ID NO: 36); IgG hinge (SEQ ID NO: 37); IgG2 transmembrane domain (SEQ ID NO: 38); FkBP12v36 (SEQ ID NO: 39); FKBP12 domain (SEQ ID NO: 40); FRB segment of mTOR (SEQ ID NO: 41); mCherry reporter (SEQ ID NO: 42); eGFP Reporter (SEQ ID NO: 43); murine CD79 α (SEQ ID NO: 44); murine CD79 β (SEQ ID NO: 45); human CD79 α (SEQ ID NO: 46); human CD79 β (SEQ ID NO: 47); linker (SEQ ID NO: 48); linker (SEQ ID NO: 49); StrepTagII (SEQ ID NO: 50); HA tag (SEQ ID NO: 51);

His tag (SEQ ID NO: 52); Avi tag (SEQ ID NO: 53); sFLAG (SEQ ID NO: 54); Tev (SEQ ID NO: 55); Flag tag (SEQ ID NO: 56); Xpress tag (SEQ ID NO: 57); Calmodulin tag (SEQ ID NO: 58); Myc tag (SEQ ID NO: 59); Softag 1 (SEQ ID NO: 60); Softag 3 (SEQ ID NO: 61); STREP® tag (SEQ ID NO: 62); and V5 tag (SEQ ID NO: 63).

DETAILED DESCRIPTION

[0024] Vaccines are designed to increase the immunity of a subject against a particular infection by stimulating B cells to produce antibodies against the targeted infectious agent. Routine pediatric vaccination is a long-established clinical intervention with comparatively low risk and high efficacy. Unfortunately, however, vaccinations are not available for all infectious agents. As one example, every year in the United States, millions of children visit a doctor or emergency room due to infections with Respiratory Syncytial Virus (RSV).

[0025] In addition to combating infections, antibodies can also be useful as treatments for other conditions such as autoimmune diseases. However, these antibody-based therapies typically require repeated injections of the antibodies to maintain protection.

[0026] Technologies have been developed to genetically modify B cells to express selected antibodies (see, e.g., WO2019/079772, which is incorporated by reference herein for its teachings regarding the same). Potent B cell responses, however, typically require these modified B cells to interact with CD4+ T cells to receive activation signals from the T cells. If the T cells have not encountered the antigen, such interactions may not sufficiently occur. Absent the required T cell interactions, B cells expressing selected antibodies may fail to adequately expand, survive, and differentiate.

[0027] B cell's natural dependence on T cell interactions also hampers the development of universal donor B cells as broadly applicable therapeutics. This is because T cells interact with B cells by binding to proteins presented on the B cell surface within major histocompatibility complex (MHC) class II molecules. In humans, MHC molecules are referred to as human leukocyte antigen (HLA) molecules. More than 600 HLA alleles are known, and more than 10,000 alleles are estimated (Lim et al., Cell. 168(4):724-740, 2017). The frequency of expression of HLA alleles varies by population and ethnic group (see, for instance, U.S. Patent No. 8,268,964). Unfortunately, the most frequent allele is only present in 10% of the population, and T cells will reject any cell that expresses a different MHC allele than the host.

[0028] The most important T cell activation signal comes through the binding of CD40 expressed by B cells with CD40L expressed by T cells. Such stimulation of CD40 by CD40L binding promotes cytokine production (e.g., IL-2, IL-4, IL-21, IL-10, IFN- γ , BAFF/BLyS), isotype switching, GC

formation, somatic hypermutation, and formation of long-lived plasma B cells and memory B cells. FIG. 1 provides a schematic depicting B-cell/T-cell interactions including CD40/CD40L binding.

[0029] CD40's binding partner, CD40L (CD154) is found mainly on activated mature T cells but also appears on granulocytes, macrophages, and platelets. CD40/CD40L binding interactions are depicted in FIG. 2. CD40 binds asymmetrically to two subunits of CD40L's trimeric complex. Though CD40L is a trimer, it does not lead to trimerization of CD40. This is evident in the 2:3 molar ratio of CD40:CD40L that is consistently isolated. CD40L is endocytosed and then degraded by the B cell once CD40 interacts with CD40L. This interaction also promotes the release of soluble CD40 which will bind to CD40L on T cells and through proteolytic cleavage down regulate the mRNA expression of CD40L. CD40 activation by its ligand activates several pathways due to phosphorylation of TRAF pathways such as NFkB, MAPK and PI3k. It is important to note that CD40 does not have a kinase domain and does not phosphorylate. TRAF5 activation is key for B cell proliferation and regulation of surface receptors. FIG. 3 depicts intracellular CD40 signaling pathways that can result in phosphorylation of p38, Erk, and Stat3, for example. Stimulation of CD40 signaling pathways can ultimately result in activation of B cell activities as described previously and as depicted in FIG. 4.

[0030] The current disclosure describes B cells that receive sufficient activation signals independently of CD40L binding. "Independently of CD40L binding" and "independently of interaction with CD40L" (used interchangeably herein) means that CD40L binding is not required for B cell activation. Because the B cells do not require CD40L binding for activation, they can be engineered into universal donor cells by deleting non-host compatible MHC molecules.

[0031] The current disclosure provides B cells that receive sufficient activation signals independently of CD40L binding by disclosing fusion proteins that allow CD40 activation signals following antigen binding to a B cell. One example of such a fusion protein includes, when expressed, an extracellular antigen binding domain linked through a transmembrane domain to an intracellular CD40 signaling domain. Examples of this approach are depicted in FIG. 5, showing fusion proteins including an scFv binding domain, one or more tag sequences, one or more spacer sequences, one or more multimerization molecules, a transmembrane domain, and an intracellular CD40 signaling domain. Such proteins can be expressed with signal peptides, reporter proteins (e.g., mCherry, truncated EGFR (tEGFR)) and skipping elements (e.g., IRES or self-cleaving peptides).

[0032] Another approach disclosed herein utilizes antigen-binding based B cell receptor (BCR) signaling to trigger CD40 signaling. BCR naturally transmit B cell activation signals through the intracellular proteins CD79 α and CD79 β when bound by an antigen (see FIG. 6A). More

particularly, BCR on B cells electrostatically associate CD79 α and CD79 β . CD79 α and CD79 β are intrinsically disordered but have a secondary structure tendency with immunoreceptor tyrosine-based activation motif (ITAM) regions for downstream molecule interactions with kinases. These proteins are phosphorylated due to crosslinking from BCR aggregation from interaction with antigen. Upon phosphorylation, both the α and β subunit change conformational shape in degree of helical turn. The α subunit increases in helicity while the β subunit loses helical tightness. The sequence between the α and β subunit are only 20% similar and the β subunits exact function has yet to be fully elucidated. The ITAM regions are highly sensitive to mutations especially in the N-terminal residues. If the regions are disrupted, calcium mobilization and phosphorylation of subsequent proteins like Syk and Src will be affected.

[0033] As shown in FIGs. 6B-6D, fusion proteins can be generated wherein the intracellular signaling portion of CD40 is linked to the intracellular signaling portion of the BCR itself. An intracellular CD40 signaling domain can be linked to a CD79 α subunit and a CD79 β subunit (FIG. 6B), a CD79 α subunit and a CD79 α subunit (FIG. 6C), or a CD79 β subunit and a CD79 β subunit (FIG. 6D). Using this second approach the antigen binds to the BCR which transmits both BCR and CD40 signals substantially simultaneously. As with other fusion proteins described herein, these embodiments can similarly be expressed with one or more signal peptides, reporter proteins (e.g., mCherry, tEGFR), skipping elements (e.g., IRES or self-cleaving peptides), and/or multimerization molecules.

[0034] CD40 intracellular signaling domains utilized within embodiments disclosed herein result in activation of the B cell when bound by antigen. The term "CD40 intracellular signaling domain" is thus meant to include any portion of the intracellular domain sufficient to transduce an activation signal. A CD40 intracellular signaling domain can directly or indirectly promote a biological or physiological response in a cell when receiving the appropriate signal. Exemplary CD40 intracellular signaling domains are provided in FIGs. 10A-10H and 11. Functional variants and fragments of fusion proteins and intracellular signaling domains may also be used. Functional variants include one or more residue additions or substitutions that do not substantially impact the physiological effects of a protein. Functional fragments include one or more deletions or truncations that do not substantially impact the physiological effects of a protein. In particular embodiments, CD40 signaling through a fusion protein disclosed herein or a functional variant or fragment thereof is evidenced by an increase in one or more of: p38 phosphorylation, Erk1/2 phosphorylation, Stat3 phosphorylation, cytokine production (e.g., IL-2, IL-4, IL-21, IL-10, IFN-g, BAFF/BLyS production), B cell proliferation, plasma cell differentiation, and antibody production.

[0035] When a subject donates his or her own B cells for genetic engineering or is otherwise

immunologically matched with a donor, no modifications regarding the B cell's MHC class II alleles are required. However, when B cells genetically engineered as disclosed herein are used as universal donor cells, MHC class II molecules should be deleted. MHC class II molecule expression can be deleted by reducing or eliminating activity of one or more transcription factors required for MHC class II molecule expression. Such transcription factors include CIITA, TRAC, TRBC, B2M, RFX5, and RFXAP. As used herein, "deleting" or "eliminating" does not require complete deletion or elimination but instead refers to reduced levels leading to clinically-acceptable use. "Clinically-acceptable use" means that research or clinical benefit outweighs potential risk, as determined by a relevant regulatory authority (e.g., IACUC or the FDA) and/or a treating researcher, physician, or veterinarian.

[0036] Aspects of the disclosure are now described with additional detail and options as follows: (i) Extracellular Antigen Binding Domains; (ii) Exemplary Anti-Viral Binding Domains; (iii) Spacers, Linkers, and Junction Amino Acids; (iv) Tags; (v) Multimerization Molecules; (vi) Transmembrane Domains; (vii) Skipping Elements; (viii) Reporters; (ix) Gene Editing Techniques and Cell Sorting; (x) Formulation of Modified B cells; (xi) Methods of Use; (xii) Exemplary Embodiments; (xiii) Experimental Examples; and (xiv) Closing Paragraphs. These headings do not limit the interpretation of the disclosure and are provided for organizational purposes only.

[0037] (i) Extracellular Antigen Binding Domains. Approaches to activate B cells through antigen binding, independently of interactions with CD40L utilize an extracellular antigen binding domain. The extracellular antigen binding domain can be an antigen binding domain such as an engineered binding domain (e.g., an engineered antibody binding domain) or a BCR.

[0038] One approach to activating CD40 signaling in B cells independently of interactions with CD40L includes expressing an engineered molecule that results in an extracellular antigen binding domain that binds an antigen of interest. In some examples, B cells expressing the engineered molecule that results in an extracellular antigen binding domain that binds an antigen of interest will also be genetically modified to express antibodies that bind the antigen of interest.

[0039] Engineered antigen binding domains can take many forms. Particular examples are binding domain fragments derived from antibody binding domains. For example, particular embodiments can include binding fragments of an antibody or engineered forms thereof, e.g., Fv, Fab, Fab', F(ab')₂, Fab'-SH, diabodies; and linear antibodies, single chain Fv fragments (scFvs) or any biologically effective fragments of an immunoglobulin that bind specifically to a targeted antigen. In other words, an "antibody fragment" denotes a portion of a complete or full-length antibody that retains the ability to bind to an epitope. Antibody fragments can be made by various techniques, including proteolytic digestion of an intact antibody as well as production by

recombinant host-cells (e.g., mammalian suspension cell lines, E. coli or phage), as described herein. Antibody fragments can be screened for their binding properties in the same manner as intact antibodies. Engineered forms of antibody binding domains or fragments thereof re-arrange or newly-construct antibody sequences or formats into non-naturally occurring forms thereof.

[0040] A single chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy and light chains of immunoglobulins connected with a short linker peptide. Fv fragments include the V_L and V_H domains of a single arm of an antibody but lack the constant regions. Although the two domains of the Fv fragment, V_L and V_H , are coded by separate genes, they can be joined, using, for example, recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (single chain Fv (scFv)). For additional information regarding Fv and scFv, see e.g., Bird, et al., Science 242:423-426, 1988; Huston, et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988; Plueckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore (eds.), Springer-Verlag, New York), (1994) 269-315; WO 1993/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458.

[0041] Linker sequences that are used to connect the V_L and V_H of an scFv are generally five to 35 amino acids in length. In particular embodiments, a V_L - V_H linker includes from five to 35, ten to 30 amino acids or from 15 to 25 amino acids. Variation in the linker length may retain or enhance activity, giving rise to superior efficacy in activity studies. Linker sequences of scFv are commonly Gly-Ser linkers, described in more detail elsewhere herein.

[0042] Commonly used flexible linkers include linker sequence with the amino acids glycine and serine (Gly-Ser linkers). In particular embodiments, the linker sequence includes sets of glycine and serine repeats such as from one to ten repeats of $(Gly_xSer_y)_n$, wherein x and y are independently an integer from 0 to 10 provided that x and y are not both 0 and wherein n is an integer of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10). Particular examples include $(Gly_4Ser)_n$ (SEQ ID NO: 65), $(Gly_3Ser)_n(Gly_4Ser)_n$ (SEQ ID NO: 66), $(Gly_3Ser)_n(Gly_2Ser)_n$ (SEQ ID NO: 67), and $(Gly_3Ser)_n(Gly_4Ser)_1$ (SEQ ID NO: 68). In particular embodiments, the linker is $(Gly_4Ser)_4$ (SEQ ID NO: 69), $(Gly_4Ser)_3$ (SEQ ID NO: 49), $(Gly_4Ser)_2$ (SEQ ID NO: 70), $(Gly_4Ser)_1$ (SEQ ID NO: 71), $(Gly_3Ser)_2$ (SEQ ID NO: 72), $(Gly_3Ser)_1$ (SEQ ID NO: 73), $(Gly_2Ser)_2$ (SEQ ID NO: 74) or $(Gly_2Ser)_1$, GGS GGGSGSG (SEQ ID NO: 75), GGS GGGSGSG (SEQ ID NO: 76), or GGS GGGSG (SEQ ID NO: 77).

[0043] For additional examples of and information regarding linkers, see Chen et al., Adv Drug Deliv Rev. 2013 Oct 15; 65(10): 1357–1369.

[0044] Additional examples of engineered antigen binding domain formats include scFv-based

grababodies and soluble VH domain antibodies. These binding domains form binding regions using only heavy chain variable regions. See, for example, Jespers et al., Nat. Biotechnol. 22:1161, 2004; Cortez-Retamozo et al., Cancer Res. 64:2853, 2004; Baral et al., Nature Med. 12:580, 2006; and Barthelemy et al., J. Biol. Chem. 283:3639, 2008.

[0045] A Fab fragment is a monovalent antibody fragment including V_L, V_H, CL and CH1 domains. A F(ab')₂ fragment is a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region. For discussion of Fab and F(ab')₂ fragments having increased in vivo half-life, see U.S. Patent 5,869,046. Diabodies include two epitope-binding sites that may be bivalent. See, for example, EP 0404097; WO1993/01161; and Holliger, et al., Proc. Natl. Acad. Sci. USA 90:6444-6448, 1993. Dual affinity retargeting antibodies (DART™; based on the diabody format but featuring a C-terminal disulfide bridge for additional stabilization (Moore et al., Blood 117:4542-51, 2011)) can also be used. Engineered antibody fragments can also include isolated CDRs. For a review of antibody fragments, see Hudson, et al., Nat. Med. 9:129-134, 2003.

[0046] (ii) Exemplary Anti-Viral Binding Domains. Engineered antigen binding domains and engineered BCR can be derived from numerous antibodies, depending on the selected antigen of interest. For example, engineered antigen binding domains and engineered BCR can be prepared from an antibody that can provide a protective effect against a pathogen or condition (e.g., autoimmune disease). In particular embodiments, the engineered antigen binding domain is derived from an anti-RSV antibody, an anti-HIV antibody, an anti-Dengue virus antibody, an anti-Bordatella pertussis antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-Clostridium difficile bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.

[0047] In particular embodiments, engineered BCR are chimeric. In particular embodiments, chimeric BCR refer to a synthetic BCR that includes: (i) at least one portion that is encoded by a B cell's endogenous genome, and (ii) at least one portion that is encoded by an inserted nucleic acid. In particular embodiments, the chimeric BCR includes an endogenous heavy chain constant domain, an exogenous immunoglobulin variable and constant light chain, and an exogenous variable heavy chain. See, for example, WO 2019/079772.

[0048] The following antibodies and sequences are useful to generate engineered antigen binding domains and/or engineered BCR with targeted binding against pathogens or antigens of interest (unless noted, Kabat numbering is intended):

[0049] An exemplary anti-RSV antibody is palivizumab, which targets the RSV fusion protein and

is used to prevent or reduce RSV infections.

[0050] In particular embodiments, an anti-RSV antibody is mouse palivizumab that includes a variable heavy chain sequence including:

QVELQESGPILQPSQTLSTLCSFSGFSLSTSGMSVGWIRQPSGEGLEWLADIWWDDKKDYN
PSLKSRLTISKDTSSNQVFLKITGVDTADTATYYCARSMITNWWYFDVWGAGTTVTVSS (SEQ ID
NO: 78); and a variable light chain sequence including:

DIQLTQSPAIMSASPGEKVTMTCSASSSVGYMHWYQQKLSTSPKLQIYDTSKLASGVPGRFSG
SGSGNSYSLTISSIAEDVATYYCFRSGSGYPFTFGGQGTKLEIK (SEQ ID NO: 79).

[0051] An additional exemplary anti-RSV antibody is human palivizumab and includes a variable light chain sequence including:

DIQMTQSPSTLSASVGDRVTITCKCQLSVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSG
SGSGTEFTLTISLQPDFATYYCFQSGSGYPFTFGGQGTKLEIKR (SEQ ID NO: 80); and a variable
heavy chain sequence including:

QVTLRESGPALVKPTQTLTLCTFSGFSLSTSGMSVGWIRQPPGKALEWLADIWWDDKKDYN
PSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWWYFDVWGAGTT (SEQ ID NO:
81).

[0052] Within a variable heavy chain and variable light chain, segments referred to as complementary determining regions (CDRs) dictate epitope binding. Each heavy chain has three CDRs (i.e., CDRH1, CDRH2, and CDRH3) and each light chain has three CDRs (i.e., CDRL1, CDRL2, and CDRL3)

[0053] An additional exemplary anti-RSV antibody is described in U.S. Patent No. 9,403,900. This anti-RSV antibody includes a variable heavy chain including a CDRH1 sequence including GASINSDNYWT (SEQ ID NO: 82), a CDRH2 sequence including HISYTGNTYYTPSLKS (SEQ ID NO: 83), and a CDRH3 sequence including CGAYVLISNCGWFDS (SEQ ID NO: 84); and a variable light chain including a CDRL1 sequence including QASQDISTYLN (SEQ ID NO: 85), a CDRL2 sequence including GASNLET (SEQ ID NO: 86), and a CDRL3 sequence including QQYQYLPYT (SEQ ID NO: 87).

[0054] Exemplary anti-RSV antibodies also include AB1128 (available from MILLIPORE) and ab20745 (available from ABCAM).

[0055] An example of an anti-HIV antibody is 10E8, which is a broadly neutralizing antibody that binds to gp41. The 10E8 anti-HIV antibody includes a variable heavy chain including a CDRH1 sequence including GFDNDNAW (SEQ ID NO: 88), a CDRH2 sequence including ITGPGEWSV (SEQ ID NO: 89), and a CDRH3 sequence including TGKYYDFWSGYPPGEEYFQD (SEQ ID NO: 90); and a variable light chain including a CDRL1 sequence including TGDLSRSHYAS (SEQ

ID NO: 91), a CDRL2 sequence including GKNNRPS (SEQ ID NO: 92), and a CDRL3 sequence including SSRDKSGSRLSV (SEQ ID NO: 93).

[0056] An additional example of an anti-HIV antibody is VRC01, which is a broadly neutralizing antibody that binds to the CD4 binding site of gp120. The VRC01 antibody includes a variable heavy chain including a CDRH1 sequence including GYEFIDCT (SEQ ID NO: 94), a CDRH2 sequence including KPRGGAVN (SEQ ID NO: 95), and a CDRH3 sequence including RGKNCDYNWDFEHW (SEQ ID NO: 96); and a variable light chain including a CDRL1 sequence including QYGS (SEQ ID NO: 97), a CDRL2 sequence including SGS, and a CDRL3 sequence including QQYEF (SEQ ID NO: 98).

[0057] Exemplary anti-HIV antibodies also include ab18633 and 39/5.4A (available from ABCAM); and H81E (available from THERMOFISHER).

[0058] An example of an anti-Dengue virus antibody is antibody 55 described in U.S. 20170233460 and includes a variable heavy chain including a CDRH1 sequence including EVQLHQSGAELVKPGASVKLSCTVSGFNIK (SEQ ID NO: 99), a CDRH2 sequence including WWKQRPEQGLEWI (SEQ ID NO: 100), and a CDRH3 sequence including ATIKADTSSNTAYLQLISLTSEDVAVYYCAF (SEQ ID NO: 101); and a variable light chain including a CDRL1 sequence including DIQMTQSPASLSVSVGETVTITC (SEQ ID NO: 102), a CDRL2 sequence including WYQQKQKGKSPQLLVY (SEQ ID NO: 103), and a CDRL3 sequence including GVPSRFSGSGSGTQYSLKINSLSQSEDFGTYYC (SEQ ID NO: 104).

[0059] An additional example of an anti-Dengue virus antibody is DB2-3 described in U.S. Patent No. 8,637,035 and includes a variable heavy chain including a CDRH1 sequence including YTFTDYAIT (SEQ ID NO: 105), a CDRH2 sequence including GLISTYYGDSFYNQKFKG (SEQ ID NO: 106), and a CDRH3 sequence including TIRDGKAMDY (SEQ ID NO: 107); and a variable light chain including a CDRL1 sequence including RSSQSLVHSNGNTYLH (SEQ ID NO: 108), a CDRL2 sequence including KVSNRFS (SEQ ID NO: 109), and a CDRL3 sequence including SQSTHVPYT (SEQ ID NO: 110). Examples of anti-Dengue virus antibodies also include ab155042 and ab80914 (both available from ABCAM).

[0060] An example of an anti-pertussis antibody is described in U.S. Patent No. 9,512,204 and includes a variable heavy chain including QVQLQQPGSELVRPGASVKLSCKASGYKFTS YWMHWWKQRPQGQGLEWIGNIFPGSGSTNYDEKFNKATLTVDTSNTAYMQLSSLTSEDSAV YYCTRWLSGAYFDYWGGTTVTVSS (SEQ ID NO: 111) and a variable light chain including QIVLTQSPALMSASPGEKVTMTCSASSSVSFMYWYQQKPRSSPKPWYLTSLNPSGVPARFSG SGSGTSYSLTISSMEAEDAATYYCQQWSSHPPTFGSGTKLEIK (SEQ ID NO: 112).

[0061] An example of an anti-hepatitis C antibody includes a variable heavy chain including a

CDRH1 sequence including SYGMHW (SEQ ID NO: 113), a CDRH2 sequence including VIWLDGSNTYYADSVKGR (SEQ ID NO: 114), and a CDRH3 sequence including ARDIFTVARGVVIYFDY (SEQ ID NO: 115); and a variable light chain including a CDRL1 sequence including RASQSVSSYLA (SEQ ID NO: 116), a CDRL2 sequence including DASNRAT (SEQ ID NO: 117), and a CDRL3 sequence including QQRSNWWT (SEQ ID NO: 118). Examples of anti-hepatitis C antibodies also include MAB8694 (available from MILLIPORE) and C7-50 (available from ABCAM).

[0062] An example of an anti-influenza virus antibody is described U.S. Patent No. 9,469,685 and includes a variable heavy chain including a CDRH1 sequence including GMTSNSLA (SEQ ID NO: 119), a CDRH2 sequence including IIPVFETP (SEQ ID NO: 120), and a CDRH3 sequence including ATSAGGIVNYYLSFNI (SEQ ID NO: 121); and a variable light chain including a CDRL1 sequence including QTITTW (SEQ ID NO: 122), a CDRL2 sequence including KTS, and a CDRL3 sequence including QQYSTYSGT (SEQ ID NO: 123). An example of an anti-influenza virus antibody also includes C102 (available from THERMOFISHER).

[0063] An exemplary anti-MPV antibody includes MPE8.

[0064] Exemplary anti-CMV antibodies includes MCMV5322A, MCMV3068A, LJP538, and LJP539. RG7667 includes a mixture of MCMV5322A and MCMV3068A while CSJ148 includes a mixture of LJP538, and LJP539. See also, for example, Deng et al., *Antimicrobial Agents and Chemotherapy* 62(2) e01108-17 (Feb. 2018); and Dole et al., *Antimicrobial Agents and Chemotherapy* 60(5) 2881-2887 (May 2016).

[0065] An example of an anti-EBV antibody includes a variable heavy chain including an AMM01 CDRH1 sequence including YTFIHFGISW (SEQ ID NO: 124), an AMM01 CDRH2 sequence including IDTNNGNTNYAQSLQG (SEQ ID NO: 125), and an AMM01 CDRH3 sequence including RALEMGHRSGFPFDY (SEQ ID NO: 126); and a variable light chain including an AMM01 CDRL1 sequence including GGHNIGAKNVH (SEQ ID NO: 127), an AMM01 CDRL2 sequence including YSDRPS (SEQ ID NO: 128), and an AMM01 CDRL3 sequence including CQVWDSGRGHPLYV (SEQ ID NO: 129).

[0066] An example of an anti-HSV antibody includes HSV8-N and MB66.

[0067] Exemplary anti-Clostridium difficile antibodies include actoxumab and bezlotoxumab. See also, for example, Wilcox et al., *N Engl J Med* 376(4) 305-317 (2017).

[0068] Numerous additional antibody sequences are available and known to those of ordinary skill in the art that can be used within the teachings of the current disclosure. Sequence information for commercially available antibodies may be found in the Drug Bank database, the CAS Registry, and/or the RSCB Protein Data Bank. Moreover, nucleic acid sequences encoding

portions of selected antibodies described herein can be easily derived by one of ordinary skill in the art.

[0069] As indicated previously, when an engineered antigen binding domain is derived from an antibody, it means that the engineered antigen binding domain includes the binding domain of an antibody. Including the binding domain of an antibody means that the engineered antigen binding domain has sufficient sequence similarity with the binding domain of the antibody that it has substantially similar binding properties. In certain examples, an engineered antigen binding domain derived from an antibody has the same CDRs of the antibody, as defined by any relevant CDR numbering scheme or prediction algorithm. Examples include Kabat et al. (1991) "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (Kabat numbering scheme); Al-Lazikani et al. (1997) J Mol Biol 273: 927-948 (Chothia numbering scheme); Maccallum et al. (1996) J Mol Biol 262: 732-745 (Contact numbering scheme); Martin et al. (1989) Proc. Natl. Acad. Sci., 86: 9268-9272 (AbM numbering scheme); North et al. (2011) J. Mol. Biol. 406(2):228-56 (North numbering scheme); Lefranc M P et al. (2003) Dev Comp Immunol 27(1): 55-77 (IMGT numbering scheme); and Honegger and Pluckthun (2001) J Mol Biol 309(3): 657-670 ("Aho" numbering scheme) and software programs such as ABodyBuilder. The boundaries of a given CDR or FR may vary depending on the scheme used for identification. In certain examples, an engineered antigen binding domain derived from an antibody has the same VL and VH chain as the antibody (i.e., 100% sequence identity) or can have up to 10 residue additions, deletions, or substitutions outside of CDR residues. In certain examples, an engineered antigen binding domain derived from an antibody has a VL with 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the VL of the antibody and a VH with 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the VH of the antibody.

[0070] (iii) Spacers, Linkers, and Junction Amino Acids. Various forms of fusion proteins described herein can include spacers, linkers, and/or junction amino acids that connect different portions of the fusion proteins.

[0071] Spacer regions are generally used to create appropriate distances and/or flexibility from other fusion protein sub-components. In particular embodiments, the length of a spacer region can be customized for binding different antigens (e.g., viral antigens).

[0072] Spacer regions typically include those having 35 to 250 amino acids, 35 to 200 amino acids, 35 to 150 amino acids, 35 to 100 amino acids, or 35 to 50 amino acids.

[0073] Exemplary spacer regions include all or a portion of an extracellular CD40 domain or an immunoglobulin hinge region. An extracellular CD40 domain or immunoglobulin hinge region may

be a wild-type extracellular CD40 domain or immunoglobulin hinge region or an altered wild-type extracellular CD40 domain or immunoglobulin hinge region. In certain embodiments, an extracellular CD40 domain or immunoglobulin hinge region is a human or murine extracellular CD40 domain or immunoglobulin hinge region. As used herein, a “wild type immunoglobulin hinge region” refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody.

[0074] An immunoglobulin hinge region may be an IgG, IgA, IgD, IgE, or IgM hinge region. An IgG hinge region may be an IgG1, IgG2, IgG3, or IgG4 hinge region. Sequences from IgG1, IgG2, IgG3, IgG4 or IgD can be used alone or in combination with all or a portion of a CH2 region; all or a portion of a CH3 region; or all or a portion of a CH2 region and all or a portion of a CH3 region.

[0075] Other examples of hinge regions that can be used as spacers in fusion proteins described herein include the hinge region present in the extracellular regions of type 1 membrane proteins, such as CD8 α , CD4, CD28 and CD7, which may be wild-type or variants thereof.

[0076] A linker can include any portion of a fusion protein that serves to connect two other subcomponents of the fusion protein. Some linkers serve no purpose other than to link components while many linkers serve an additional purpose. As used herein, linkers are shorter than spacers (i.e., less than 35 amino acid residues).

[0077] Linkers can be flexible, rigid, or semi-rigid, depending on the desired function of the linker. Gly-Ser linkers are described above in relation to scFv. In this scenario, Gly-Ser linkers provide flexibility and room for conformational movement between different components of fusion proteins. Linkers may also be Gly linkers (e.g., Gly₄₋₂₀ including particularly Gly₄, Gly₅, Gly₆, Gly₇, and Gly₈).

[0078] In some situations, flexible linkers may be incapable of maintaining a distance or positioning of fusion proteins needed for a particular use. In these instances, rigid or semi-rigid linkers may be useful. Examples of rigid or semi-rigid linkers include proline-rich linkers. In particular embodiments, a proline-rich linker is a peptide sequence having more proline residues than would be expected based on chance alone. In particular embodiments, a proline-rich linker is one having at least 30%, at least 35%, at least 36%, at least 39%, at least 40%, at least 48%, at least 50%, or at least 51% proline residues. Particular examples of proline-rich linkers include fragments of proline-rich salivary proteins (PRPs).

[0079] Linkers can be susceptible to cleavage (cleavable linker), such as, acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide

bond cleavage. Alternatively, linkers can be substantially resistant to cleavage (e.g., stable linker or noncleavable linker). In some aspects, the linker is a procharged linker, a hydrophilic linker, or a dicarboxylic acid-based linker.

[0080] Junction amino acids can be a linker which can be used to connect sequences when the distance provided by a spacer region or larger linker is not needed and/or wanted. For example, junction amino acids can be short amino acid sequences that can be used to connect intracellular signaling components to tags, when present. In particular embodiments, junction amino acids are 3 amino acids or less. In particular embodiments, a glycine-serine doublet can be used as a suitable junction amino acid linker. In particular embodiments, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable junction amino acid.

[0081] (iv) Tags. Tags can be used for multiple purposes in the fusion proteins described herein. In some embodiments, tags are used during cell manufacturing for the purposes of cell isolation or tracking. Tags also have in vivo uses, for example, for tracking, activating, or depleting cells once administered.

[0082] Exemplary tags include e.g., Strep tag (e.g., the original STREP® tag, STREP® tag II, or any variant thereof; see, e.g., U.S. Patent No. 7,981,632), His tag, Flag tag, Xpress tag, Avi tag, Calmodulin tag, Polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, SBP tag, Softag 1, Softag 3, V5 tag, CREB-binding protein (CBP), glutathione S-transferase (GST), maltose binding protein (MBP), Thioredoxin tag, or any combination thereof. Antibodies and binding domains to these tags are commercially available.

[0083] Tags may be present in multiple copies in fusion proteins. For example, a fusion protein can have one, two, three, four or five tags (e.g., Strep tag). In certain embodiments, a fusion protein can include one tag, two tags, three tags, four tags, or five tags. Each of the plurality of tags may be the same or different. Exemplary embodiments include a fusion protein having a Strep tag and an HA tag, a His tag and a Strep tag, or a Myc tag and a Strep tag. Alternatively, a fusion protein can have multiple tags of the same type or same amino acid sequence, such as two, three, four or five Strep tags (e.g., Strep tag II).

[0084] Tag binding molecules (e.g., receptor, protein, antibody) may be soluble, part of a matrix composition, or conjugated to a solid surface (e.g., plate, bead). Exemplary solid surfaces include beads and particles (e.g., micro and nano), such as magnetic beads and particles.

[0085] (v) Multimerization Molecules. In certain instances, it can be beneficial to include multimerization molecules within the disclosed fusion proteins. In particular embodiments, the multimerization molecules can be part of a chemically-induced dimerization (CID) system. These systems require that one fusion protein includes a chemical inducer of dimerization binding

domain 1 (CBD1) and a second molecule (e.g., second fusion protein) includes a second chemical inducer of dimerization binding domain (CBD2), wherein CBD1 and CBD2 are capable of simultaneously binding to the chemical inducer of dimerization (CID). If the CID is rapamycin, CBD1 and CBD2 can be the rapamycin binding domain of FK-binding protein 12 (FKBP12) (SEQ ID NO: 40) and the FKBP12-Rapamycin Binding (FRB) domain of mTOR (SEQ ID NO: 41). If the CID is FK506/cyclosporin fusion protein or a derivative thereof, CBD1 and CBD2 can be the FK506 (Tacrolimus) binding domain of FK-binding protein 12 (FKBP12) and the cyclosporin binding domain of cyclophilin A. If the CID is estrone/biotin fusion protein or a derivative thereof, CBD1 and CBD2 can be an oestrogen-binding domain (EBD) and a streptavidin binding domain. If the CID is dexamethasone/methotrexate fusion molecule or a derivative thereof, CBD1 and CBD2 can be a glucocorticoid-binding domain (GBD) and a dihydrofolate reductase (DHFR) binding domain. If the CID is O⁶-benzylguanine derivative/methotrexate fusion molecule or a derivative thereof, CBD1 and CBD2 can be an O⁶-alkylguanine-DNA alkyltransferase (AGT) binding domain and a dihydrofolate reductase (DHFR) binding domain. If the CID is RSL1 or a derivative thereof, CBD1 and CBD2 can be a retinoic acid receptor domain and an ecodysone receptor domain. If the CID is AP1903 or a derivative thereof, CBD1 and CBD2 can be the FK506 binding protein (FKBP12) binding domains including a F36V mutation. Use of the CID binding domains can also be used to alter the affinity to the CID. For instance, altering amino acids at positions 2095, 2098, and 2101 of FRB can alter binding to Rapamycin: KTW has high, KHF intermediate and PLW is low (Bayle et al, Chemistry & Biology 13, 99-107, January 2006).

[0086] Examples of dimerization molecules that do not rely on CID include protein sequence motifs such as coiled coils, acid patches, zinc fingers, calcium hands, a CH1-CL pair, an "interface" with an engineered "knob" and/or "protruberance" (US 5821333), leucine zippers (US 5932448), SH2 and SH3 (Vidal et al., Biochemistry, 43:7336- 44, 2004), PTB (Zhou et al., Nature, 378:584- 592, 1995), WW (Sudol Prog Biochys MoL Bio, 65:113-132, 1996), PDZ (Kim et al., Nature, 378: 85-88, 1995; Komau et al., Science, 269:1737-1740, 1995) and WD40 (Hu et al., J Biol Chem., 273:33489- 33494, 1998). Additional examples of molecules that contain dimerization domains/motifs are receptor dimer pairs such as the interleukin-8 receptor (IL-8R), integrin heterodimers such as LFA-I and GPIIb/IIIa, dimeric ligand polypeptides such as nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, PDGF members, and brain-derived neurotrophic factor (BDNF) (Arakawa et al., J Biol. Chem., 269:27833-27839, 1994; Radziejewski et al., Biochem, 32: 1350, 1993) and variants of some of these domains with modified affinities (PCT Publication No. WO 2012/001647).

[0087] (vi) Transmembrane Domains. Particular embodiments disclosed herein include a transmembrane domain. As indicated, transmembrane domains within fusion proteins serve to connect the extracellular component and intracellular component through the cell membrane. The transmembrane domain can anchor the expressed molecule in the modified cell's membrane.

[0088] In particular embodiments, a transmembrane domain has a three-dimensional structure that is thermodynamically stable in a cell membrane, and generally ranges in length from 15 to 30 amino acids. The structure of a transmembrane domain can include an α helix, a β barrel, a β sheet, a β helix, or any combination thereof.

[0089] The transmembrane domain can be derived either from a natural and/or a synthetic source. When the source is natural, the transmembrane domain can be derived from any membrane-bound or transmembrane protein. Transmembrane domains can include at least the transmembrane region of CD40. Transmembrane domains can also be derived from transmembrane region(s) of CD2, CD3, CD4, CD5, CD8, CD9, CD11a, CD16, CD18, CD19, CD22, CD27, CD28, CD29, CD33, CD37, CD45, CD49a, CD49d, CD49f, CD64, CD80, CD84, CD86, CD96, CD100, CD103, CD134, CD137, CD150, CD154, CD160, CD162, CD226, CD229, CD244, CD278, KIRDS2, OX40, LFA-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, IL2R β , IL2R γ , IL7R α , ITGA1, VLA1, ITGA4, IA4, ITGA6, VLA-6, ITGAD, ITGAE, ITGAL, ITGAX, ITGB1, ITGB2, ITGB7, TNFR2, CEACAM1, CRT AM, PSGL1, SLAMF6 (NTB-A, Lyl08), BLAME (SLAMF8), LTBR, PAG/Cbp, NKG2D, or NKG2C. In particular embodiments, a variety of human hinges can be employed as well including the human Ig (immunoglobulin) hinge (e.g., an IgG4 hinge, an IgD hinge), a GS linker (e.g., a GS linker described herein), a KIR2DS2 hinge or a CD8a hinge.

[0090] A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid within the extracellular region of the fusion protein (e.g., up to 15 amino acids of the extracellular region) and/or one or more additional amino acids within the intracellular region of the fusion protein (e.g., up to 15 amino acids of the intracellular components). In one aspect, the transmembrane domain is from the same protein as the signaling domain (i.e., is derived from CD40). In another aspect, the transmembrane domain is derived from a protein that is not otherwise represented in any other segment of the fusion protein.

[0091] (vii) Skipping Elements. In any of the embodiments described herein, a nucleic acid can include a polynucleotide that encodes or includes a skipping element. In certain examples, the skipping element is placed between the polynucleotide segment encoding the portion of the fusion protein including the CD40 signaling domain and the polynucleotide segment encoding a reporter.

[0092] Exemplary skipping elements include self-cleaving peptides, such as the self-cleaving “2A” peptides. 2A peptides function by causing the ribosome to skip the synthesis of a peptide bond at a defined location, leading to production of two proteins from one mRNA. The 2A sequences are short (e.g., 20 amino acids), facilitating use in size-limited constructs, and proteins are produced at a 1:1 ratio. Exemplary self-cleaving 2A peptides include and/or are derived from porcine teschovirus-1 (P2A (GSG)ATNFSLLKQAGDVEENPGP (SEQ ID NO: 130)), Thosea asigna virus (T2A (GSG)EGRGSLTTCGDVEENPGP (SEQ ID NO: 131)), equine rhinitis A virus (E2A (GSG)QCTNYALLKLAGDVES NPGPP (SEQ ID NO: 132)), foot-and-mouth disease virus (F2A (GSG)VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 133)), or variants thereof. Further exemplary nucleic acid and amino acid sequences of 2A peptides are set forth in, for example, Kim et al. (PLOS One 6:e18556 (2011)).

[0093] Internal ribosome entry site (IRES) sequences can also be used as skipping elements. IRES are non-coding structured RNA sequences that allow ribosomes to initiate translation at a second internal site on a mRNA molecule, leading to production of two proteins from one mRNA.

[0094] (viii) Reporters. As indicated, fusion proteins disclosed herein can include reporters, that, in certain instances are separated from the majority of the fusion protein after expression. Exemplary reporter genes/proteins include fluorescent proteins such blue fluorescent proteins (e.g. eBFP, eBFP2, Azurite, mKalama1, GFPuv, Sapphire, T-sapphire); cyan fluorescent proteins (e.g. eCFP, Cerulean, CyPet, AmCyanI, Midoriishi-Cyan, mTurquoise); green fluorescent proteins (e.g. GFP, GFP-2, tagGFP, turboGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green (mAzamigreen), CopGFP, AceGFP, avGFP, ZsGreenI, Oregon Green™(Thermo Fisher Scientific)); Luciferase; orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato, dTomato); red fluorescent proteins (mKate, mKate2, mPlum, DsRed monomer, mCherry, mRuby, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedI, AsRed2, eqFP611, mRaspberry, mStrawberry, Jred, Texas Red™ (Thermo Fisher Scientific)); far red fluorescent proteins (e.g., mPlum and mNeptune); yellow fluorescent proteins (e.g., YFP, eYFP, Citrine, SYFP2, Venus, YPet, PhiYFP, ZsYellowI); and tandem conjugates. EGFR and truncated EGFR (tEGFR) can also be used.

[0095] (ix) Genetic Modification and Cell Sorting. Any technique known to those of ordinary skill in the art can be utilized to introduce nucleic acids encoding fusion proteins into B cells for expression.

[0096] Coding sequences encoding particular examples of fusion proteins described herein are provided in FIGs. 10A-10H and 11. To the extent not explicitly disclosed, coding sequences can be obtained from publicly available databases, publications, and the knowledge of those or

ordinary skill in the art. Coding sequences can further include sequence variants wherein the alterations do not affect (or significantly affect) the function of the encoded molecule. In certain examples, sequence variants are due to different codon optimization strategies. The terms “code”, “coding”, “encode” and “encoding” refer to a property of sequences of nucleic acids, such as a vector, a plasmid, a gene, cDNA, or mRNA, to serve as templates for synthesis of other molecules such as proteins.

[0097] Nucleic acids may include genes. The term “gene” may include not only coding sequences but also regulatory regions such as promoters, enhancers, insulators, and/or post-regulatory elements, such as termination regions.

[0098] In particular embodiments, nucleic acids including genes are provided to B cells within vectors. The term vector refers to a nucleic acid molecule capable of transferring or transporting another nucleic acid molecule, such as a gene. A vector may include sequences that direct autonomous replication in a cell or may include sequences that permit integration into host cell DNA. Useful vectors include, for example, plasmids (e.g., DNA plasmids or RNA plasmids), transposons, cosmids, bacterial artificial chromosomes, and viral vectors. Each of these may be used to deliver nucleic acids to B cells.

[0099] Gene editing systems allow control over target sites for genetic insertions or deletions in the genome. Within the teachings of the current disclosure, any gene editing system capable of precise sequence targeting and modification can also be used. These systems typically include a targeting element for precise targeting and a cutting element for cutting the targeted genetic site. Guide RNA is one example of a targeting element while various nucleases provide examples of cutting elements. Targeting elements and cutting elements can be separate molecules or linked, for example, by a nanoparticle. Alternatively, a targeting element and a cutting element can be linked together into one dual purpose molecule. When insertion of a therapeutic nucleic acid sequence is intended, the systems can also include homology-directed repair templates (i.e., homology arms as described above) associated with the nucleic acid intended for insertion.

[0100] Particular embodiments utilize zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), MegaTALs, and/or CRISPR-based systems for genome editing.

[0101] For information regarding ZFNs, see Kim, et al. Proceedings of the National Academy of Sciences of the United States of America 93, 1156-1160 (1996); Wolfe, et al. Annual review of biophysics and biomolecular structure 29, 183-212 (2000); Bibikova, et al. Science 300, 764 (2003); Bibikova, et al. Genetics 161, 1169-1175 (2002); Miller, et al. The EMBO journal 4, 1609-1614 (1985); and Miller, et al. Nature biotechnology 25, 778-785 (2007).

[0102] For information regarding TALENs, see Boch, et al. Science 326, 1509-1512 (2009);

Moscou, & Bogdanove, Science 326, 1501 (2009); Christian, et al. Genetics 186, 757-761 (2010); and Miller, et al. Nature biotechnology 29, 143-148 (2011).

[0103] The CRISPR nuclease system is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. Within CRISPR systems, a nuclease enzyme can be programmed by a short guide RNA (gRNA) molecule to recognize a specific DNA target. Within CRISPR systems, gRNA can be referred to as crRNA.

[0104] At least three different Cas9 nucleases have been developed for genome editing. The first is the wild type Cas9 which introduces double strand breaks (DSBs) at a specific DNA site, resulting in the activation of DSB repair machinery. DSBs can be repaired by non-homologous end joining (NHEJ), homology-directed repair (HDR), or microhomology mediated repair (MMEJ). NHEJ can involve repair of a DSB with no homology (<5 bp) between the two ends joined during repair; HDR can involve repair of a DSB with a large region of homology between the ends joined during repair (100 or more nucleotides); and MMEJ can involve repair of a DSB with a small (5 to 50 bp) region of homology between the ends joined during repair. Another type of Cas9 includes a mutant Cas9, known as the Cas9D10A, with only nickase activity, which means that it only cleaves one DNA strand and does not activate NHEJ. Thus, the DNA repairs proceed via the HDR pathway only. The third is a nuclease-deficient Cas9 (dCas9) which does not have cleavage activity but is able to bind DNA. Therefore, dCas9 is able to target specific sequences of a genome without cleavage. By fusing dCas9 with various effector domains, dCas9 can be used either as a gene silencing or activation tool.

[0105] Cpf1 is another nuclease suitable for use within CRISPR-based systems (see Zetsche et al. (2015) Cell 163(3): 759-771). The Cpf1 nuclease particularly can provide added flexibility in target site selection by means of a short, three base pair recognition sequence (TTN), known as the protospacer-adjacent motif or PAM. Cpf1's cut site is at least 18bp away from the PAM sequence, thus the enzyme can repeatedly cut a specified locus after indel (insertion and deletion) formation, increasing the efficiency of HDR. Moreover, staggered DSBs with sticky ends permit orientation-specific donor template insertion.

[0106] Additional information regarding CRISPR-Cas systems and components thereof are described in, US8697359, US8771945, US8795965, US8865406, US8871445, US8889356, US8889418, US8895308, US8906616, US8932814, US8945839, US8993233 and US8999641 and applications related thereto; and WO2014/018423, WO2014/093595, WO2014/093622, WO2014/093635, WO2014/093655, WO2014/093661, WO2014/093694, WO2014/093701, WO2014/093709, WO2014/093712, WO2014/093718, WO2014/145599, WO2014/204723,

WO2014/204724, WO2014/204725, WO2014/204726, WO2014/204727, WO2014/204728, WO2014/204729, WO2015/065964, WO2015/089351, WO2015/089354, WO2015/089364, WO2015/089419, WO2015/089427, WO2015/089462, WO2015/089465, WO2015/089473 and WO2015/089486, WO2016205711, WO2017/106657, WO2017/127807 and applications related thereto.

[0107] In particular embodiments, nucleotide segments encoding scFv or CD40-CD79 α/β linked molecules can be inserted into the light chromosome locus, for example, to knockout endogenous expression.

[0108] When genetic modifications reduce expression of a transcription factor to delete MHC class II molecules, gRNA molecules for CIITA deletion include: UCGAGUUGGAUGUGGAAGGU (SEQ ID NO: 134), UUUUCAUCCCCACUUCACAC (SEQ ID NO: 135), CCUCGGGGGAGAGAGAGGUG (SEQ ID NO: 136), UGGGCUCAGGUGCUUCCUCA (SEQ ID NO: 137), UCAAAGUAGAGCACAUAGGA (SEQ ID NO: 138), CCAUCAAAAGUCCUUUUUGG (SEQ ID NO: 139), GUGUCUACACUAGCCUUUC (SEQ ID NO: 140), GGGUGAAAUUCCCAACUUU (SEQ ID NO: 141), CCGGCCUUUUUACCUUGGGG (SEQ ID NO: 142), and UCUGCAGCCUUCCCAGAGGA (SEQ ID NO: 143). An sgRNA sequence for CIITA deletion is also provided in FIG. 9 as SEQ ID NO: 1.

[0109] gRNA molecules for TRAC deletion include: UUGCUCAGGCCACAGCACU (SEQ ID NO: 144), UCGACCAGCUUGACAUCACA (SEQ ID NO: 145), AGAAUCAAAAUCGGUGAAUA (SEQ ID NO: 146), CAUGUGCAAACGCCUUAAC (SEQ ID NO: 147), AAAGUUUAGGUUCGUAUCUG (SEQ ID NO: 148), UUUGAGAAUCAAAAUCGGUG (SEQ ID NO: 149), AUUCUCAAACAAAUGUGUCA (SEQ ID NO: 150), CUUUUAGAAAGUCCUGUGA (SEQ ID NO: 151), AAAGCUUUUCUGCACCAGCU (SEQ ID NO: 152), and GAGUCUCUCAGCUGGUACAC (SEQ ID NO: 153).

[0110] gRNA molecules for TRBC deletion include: CAGAGGACCUGAAAAACGUG (SEQ ID NO: 154), AGGUCCUCUGGAAAGGGAAG (SEQ ID NO: 155), AGCCAUCAGAAGCAGAGAUC (SEQ ID NO: 156), GGUGUGGGAGAUCUCUGCUU (SEQ ID NO: 157), GCCCUAUCCUGGGUCCACUC (SEQ ID NO: 158), UUCCCCUGUUUUCUUUCAGA (SEQ ID NO: 159), UUUCAGACUGUGGCUUCACC (SEQ ID NO: 160), AGGCCUCGGCGCUGACGAUC (SEQ ID NO: 161), CAGGCCCCACUCACCUGCUC (SEQ ID NO: 162), and AGGCCCCACUCACCUGCUCU (SEQ ID NO: 163).

[0111] gRNA molecules for B2M deletion include: UGGCCUGGAGGCUAUCCAGC (SEQ ID NO: 164), CCGAUAUUCCUCAGGUACUC (SEQ ID NO: 165), GAGUACCUGAGGAAUAUCGG (SEQ ID NO: 166), CUCACGUCAUCCAGCAGAGA (SEQ ID NO: 167),

CAUUCUCUGCUGGAUGACGU (SEQ ID NO: 168), ACUUUCCAUUCUCUGCUGGA (SEQ ID NO: 169), CUGAAUUGCUAUGUGUCUGG (SEQ ID NO: 170), AUCCAUCCGACAUGAAGUU (SEQ ID NO: 171), AAUUCUCUCUCCAUUCUUA (SEQ ID NO: 172), and AGCAAGGACUGGUCUUUCUA (SEQ ID NO: 173). An sgRNA sequence for B2M deletion is also provided in FIG. 9 as SEQ ID NO: 2.

[0112] For additional information regarding gRNAs to delete genes encoding CIITA, TRAC, TRBC, and B2M, see US 2020/0299661.

[0113] gRNA molecule for RFX5 deletion include:

AAAAUGUUUUAUGUAUAUGUGUUA (SEQ ID NO: 174),
 AAUGUUUUAUGUAUAUGUGUUAU (SEQ ID NO: 175),
 UAUUAUAAUUUCUAAAAUCUGCUG (SEQ ID NO: 176),
 UAUAAUUUCUAAAAUCUGCUGUGG (SEQ ID NO: 177),
 UCCCUGAAAGAAAAUCAGUGUUUCU (SEQ ID NO: 178),
 CCCUGAAAGAAAAUCAGUGUUUCUU (SEQ ID NO: 179),
 CCCUGACAGAAGCUACCCUAUGUAG (SEQ ID NO: 180),
 AGCUACCCUAUGUAGAGGACAAAGU (SEQ ID NO: 181),
 AAGUAGGUCUCAAUAAAUUUAGU (SEQ ID NO: 182), and
 AUUAGUUGGUUUACUGCUUUUCCCA (SEQ ID NO: 183).

[0114] gRNA molecules for RFXAP deletion include:

CGCGGGCGUACACACGCUGACGCGC (SEQ ID NO: 184),
 CGUACACACGCUGACGCGCAGGCUG (SEQ ID NO: 185),
 GCUGACGCGCAGGCUGCGGUCGCGC (SEQ ID NO: 186),
 CAGGCUGCGGUCGCGCAGGCGCAGU (SEQ ID NO: 187),
 GGCUGCGGUCGCGCAGGCGCAGUCG (SEQ ID NO: 188),
 CGCAGGCGCAGUCGGGGCGCCUCC (SEQ ID NO: 189),
 CGCAGUCGGGGCGCCUCCCCGUUAU (SEQ ID NO: 190),
 UUUACCCCAGCGUGUCCUGAGUCUU (SEQ ID NO: 191), and
 AGUCUUUGGUUCGCGAAGUGCCGUU (SEQ ID NO: 192).

[0115] For additional information regarding gRNAs to delete genes encoding RFX5 and RFXAP, see PCT/US2019/036,111.

[0116] gRNA sequences for RFXANK deletion include:

CGGCTCCTCCATAGCGACCG (SEQ ID NO: 193), CCTCCATAGCGACCGCGGCC (SEQ ID NO: 194), GCGACCGCGGCCGAGCGAAC (SEQ ID NO: 195), CGACCGCGGCCGAGCGAACC (SEQ ID NO: 196), CGGCCGAGCGAACCGGGAAA (SEQ ID NO: 197),

AGCGAACCGGGAAACGGCAC (SEQ ID NO: 198), GAACCGGGAAACGGCACCGG (SEQ ID NO: 199), GGGAAACGGGCACCGGAAGCC (SEQ ID NO: 200), GAAACGGGCACCGGAAGCCCG (SEQ ID NO: 201), and AAACGGGCACCGGAAGCCCGG (SEQ ID NO: 202).

[0117] Additional gRNA molecules and targeting domains for RFXANK can be found in US 2019/0309259.

[0118] In particular embodiments, nucleic acids, and/or genome targeting and cutting elements can be administered through electroporation, nanoparticle-mediated delivery and/or viral vector delivery. Adeno-associated viral vectors include those derived from e.g., adenovirus 5 (Ad5), adenovirus 35 (Ad35), adenovirus 11 (Ad11), adenovirus 26 (Ad26), adenovirus 48 (Ad48) or adenovirus 50 (Ad50)), and adeno-associated virus (AAV; see, e.g., U.S. Pat. No. 5,604,090; Kay et al., *Nat. Genet.* 24:257 (2000); Nakai et al., *Blood* 91:4600 (1998)).

[0119] In particular embodiments, genome targeting and cutting elements can be administered through electroporation and nucleic acids for insertion can be administered through AAV-mediated delivery. In particular embodiments, genome targeting and cutting elements can be administered through nanoparticle-mediated delivery and nucleic acids for insertion can be administered through AAV-mediated delivery.

[0120] In particular embodiments, a nucleic acid for delivery to B cells can be mixed with a targeting element (e.g., gRNA) and a cutting element (e.g., Cas9 or cpf1) immediately or shortly before electroporation. Selected fusion protein expression can be confirmed later (e.g., 3 days later) by measuring cell binding to fluorescently tagged target proteins by flow cytometry. Enrichment and analysis methodologies for detecting and analyzing epitope-specific B cells can be used. Pape et al., *Science*. 2011;331(6021):1203-7; Taylor et al., *J Exp Med*. 2012;209(3):597-606; Taylor et al., *J Exp Med*. 2012;209(11):2065-77; Haasken et al., *J Immunol*. 2013;191(3):1055-62; Taylor et al., *J Immunol Methods*. 2014;405:74-86; Nanton et al., *Eur J Immunol*. 2015;45(2):428-41; Hamilton et al., *J Immunol*. 2015;194(10):5022-34; Taylor et al., *Science*. 2015;347(6223):784-7).

[0121] In particular embodiments, cells can be identified and/or sorted based on marker expression, before or after delivering the nucleic acids. For example, it may be useful to isolate a particular type of B cells (e.g., memory B cells, antibody-secreting B cells, naïve B cells, B1 B cells, marginal zone B cells) from a sample prior to delivering nucleic acids. As another example, it may be useful to isolate B cells from other cells present in a blood sample. CD19 is an example of a protein expressed by B cells but few other cells of the body. By marking CD19 with a fluorescent molecule, B cells can be specifically identified. B220 is a useful marker to identify mouse B cells.

[0122] CD27 is an example of a protein expressed by memory but not naive human B cells. By marking CD27 with a fluorescent molecule, memory B cells can be identified.

[0123] CD21 is an example of a protein not expressed (or expressed to a low degree) by some memory human B cells with the capacity to quickly secrete antibody following infection. Low CD21 expression can be used to define B cells primed for plasma cell differentiation. By marking CD21 with a fluorescent molecule, these B cells can be specifically identified by for example, negative selection.

[0124] Human naïve B cells can be identified by the marker profile IgM+ IgD+ CD27-. Mouse naïve B cells can be identified by the marker profile CD38+ GL7- IgM+ IgD+. Human B1 B cells can be identified by the marker profile CD5+ CD43+. Mouse B1 B cells can be identified by the marker profile CD43+ B220LOW. Human marginal zone B cells can be identified by the marker profile CD21+++ IgM++ IgD- CD27+. Mouse marginal zone B cells can be identified by the marker profile CD21+++ IgM++ IgD-.

[0125] Particular embodiments may utilize the CD19⁺CD27⁺CD21^{lo} marker profile.

[0126] As indicated, in particular embodiments, cells may be identified and/or isolated using flow cytometry. Flow cytometry is a sensitive and powerful analysis approach that uses lasers to individually analyze the fluorescent molecules marking millions of individual cells. By analyzing the combination of fluorescent molecules each cell is marked with, different B cell subtypes can be identified.

[0127] In particular embodiments, methods of modifying B cells can include obtaining hematopoietic stem cells (HSC), and/or delivering the nucleic acids to HSC. HSC can refer to a type of stem cell that naturally produces B cells as well as all other cells of the immune system. HSC can be obtained, for example, from cord blood.

[0128] In particular embodiments, B cells may be obtained from a human subject and obtained B cells or a subset thereof may be modified ex vivo.

[0129] (x) Formulations of Modified B Cells. Once modified, B cells can be harvested from a culture medium, and washed and concentrated into a carrier in a therapeutically-effective amount. Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Nonnosol-R (Abbott Labs), PLASMA-LYTE A® (Baxter Laboratories, Inc., Morton Grove, IL), glycerol, ethanol, and combinations thereof.

[0130] In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% hyaluronic acid sodium salt (HAS) or dextrose. Additional isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar

alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0131] Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0132] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

[0133] Where necessary or beneficial, formulations can include a local anesthetic such as lidocaine to ease pain at a site of injection.

[0134] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0135] Formulations can include, for example, greater than 10^2 modified B cells, greater than 10^3 modified B cells, greater than 10^4 modified B cells, greater than 10^5 modified B cells, greater than 10^6 modified B cells, greater than 10^7 modified B cells, greater than 10^8 modified B cells, greater than 10^9 modified B cells, greater than 10^{10} modified B cells, or greater than 10^{11} modified B cells.

[0136] B cells formulation can be allogeneic or autologous to a subject, depending on whether the B cells were derived from the subject and/or whether MHC class II molecules have been deleted from the B cells.

[0137] (xi) Methods of Use. Methods disclosed herein include treating subjects (e.g., humans, veterinary animals (dogs, cats, reptiles, birds) livestock (e.g., horses, cattle, goats, pigs, chickens) and research animals (e.g., monkeys, rats, mice, fish) with formulations disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic

treatments.

[0138] An "effective amount" is the amount of a composition necessary to result in a desired physiological change in the subject. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically-significant effect in an animal model or in vitro assay relevant to the assessment of a condition's development, progression, and/or resolution.

[0139] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a condition or displays only early signs or symptoms of a condition such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the condition. Thus, a prophylactic treatment functions as a preventative treatment against a condition. In particular embodiments, prophylactic treatments reduce, delay, or prevent the worsening of a condition. Particular embodiments include administration of a formulation described herein as prophylactic protection in the absence of a currently effective vaccine. Particular embodiments include administration of a formulation described herein as prophylactic protection as a replacement for conventional vaccination strategies. Particular embodiments include administration of a formulation described herein as prophylactic protection as a supplement to conventional vaccination strategies.

[0140] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a condition and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the condition. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the condition and/or reduce control or eliminate side effects of the condition.

[0141] In particular embodiments, the condition is an infection.

[0142] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0143] In particular embodiments, therapeutically effective amounts provide anti-pathogen effects. Anti-pathogen effects can include anti-infection effects. Anti-infection effects can include a decrease in the occurrence of infections, a decrease in the severity of infections, a decrease in the duration of infections, a decrease in the number of infected cells, a decrease in volume of infected tissue, an increase in life expectancy, induced sensitivity of infected cells to immune clearance, reduced infection-associated pain, and/or reduction or elimination of a symptom associated with the treated infection.

[0144] In particular embodiments, therapeutically effective amounts provide anti-inflammatory

effects. Anti-inflammatory effects can include reduced inflammation-associated pain, heat, redness, swelling and/or loss of function.

[0145] In particular embodiments, therapeutically effective amounts provide anti-Crohn's disease effects or anti-ulcerative colitis effects. Anti-Crohn's disease effects or anti-ulcerative colitis effects can include reduced diarrhea, reduced rectal bleeding, reduced unexplained weight loss, reduced fever, reduced abdominal pain and cramping, reduced fatigue and feelings of low energy, and/or restored appetite.

[0146] In particular embodiments, therapeutically effective amounts provide anti-arthritis effects. Anti-arthritis effects can include reduced pain, stiffness, swelling, redness in the joints and/or a restored range of motion. Types of arthritis include rheumatoid arthritis (RA), ankylosing spondylitis, and psoriatic arthritis.

[0147] In particular embodiments, therapeutically effective amounts provide anti-plaque psoriasis effects. Anti-plaque psoriasis effects can include reduced red patches, scaling spots, itching, burning, soreness, nail bed abnormalities and/or swollen or stiff joints.

[0148] In particular embodiments, B cells may be obtained, a subset of the B cells may be modified ex vivo, and then the modified B cells may be formulated and administered a subject. Obtained B cells can be modified to express a fusion protein that allows CD40 signaling and activation independently of interactions with CD40L. Obtained B cells can also optionally be modified to delete MHC class II molecule expression.

[0149] In particular embodiments, a first set of B cells may be modified with nucleic acids to produce a selected antibody against a first pathogen, and a second set of B cells may be modified with different nucleic acids to produce a selected antibody against a second pathogen, thereby providing protective antibodies against two pathogens. The B cells may similarly be modified to express fusion proteins disclosed herein having engineered antigen binding domains that bind the same pathogens to trigger B cell activation independently of interactions with CD40L. Additionally or alternatively, the B cells may be modified to express fusion proteins linking CD40 signaling to CD79 α and/or CD79 β domains.

[0150] B cells against any number of pathogens can be formed and administered to a subject. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-HIV antibody, an anti-Dengue virus antibody, an anti-Bordatella pertussis antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-MPV antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-Clostridium difficile bacterial toxin antibody, and/or an anti-TNF antibody. In particular embodiments, the selected antibodies can be one or more of an anti-RSV

antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and/or an anti-MPV antibody. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and an anti-MPV antibody. In particular embodiments, the selected antibody is palivizumab. The B cells may similarly be modified to express fusion proteins disclosed herein having engineered antigen binding domains that bind the same pathogens to trigger B cell activation independently of interactions with CD40L. Additionally or alternatively, the B cells may be modified to express fusion proteins linking CD40 signaling to CD79 α and/or CD79 β domains.

[0151] In particular embodiments, B cells may be obtained from a bone marrow donor or a hematopoietic stem cell donor that has been immunologically matched to a recipient. As indicated, obtained B cells can be modified to express a fusion protein that allows CD40 activation independently of interactions with CD40L. In particular embodiments, a first subset of the donor's B cells may be modified with nucleic acids to produce a selected antibody against a first pathogen, and a second subset of the donor's B cells may be modified with different nucleic acids to produce a selected antibody against a second pathogen, thereby providing protective antibodies against two pathogens. The genetically-modified B cells can be administered to the recipient to provide protection against infection (e.g., an anti-infection effect) until the transplanted cells repopulate the recipient's own immune system. The administered B cells may similarly be modified to express fusion proteins disclosed herein having engineered antigen binding domains that bind the same pathogens to trigger B cell activation independently of interactions with CD40L. Additionally or alternatively, the B cells may be modified to express fusion proteins linking CD40 signaling to CD79 α and/or CD79 β domains.

[0152] In particular embodiments, the recipient is receiving bone marrow from a donor or a hematopoietic stem cell transplant as a treatment for a hematological malignancy. Examples of hematological malignancies include acute lymphocytic leukemia, B-cell prolymphocytic leukemia, Burkitt lymphoma/leukemia, chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma, follicular lymphoma (grades I, II, III, or IV), Hodgkin's lymphoma, intravascular large B-cell lymphoma, lymphoma, lymphoplasmocytic lymphoma, mantle cell lymphoma, marginal zone lymphoma (extra-nodal and nodal), mediastinal (thymic) large B-cell lymphoma, multiple myeloma, non-Hodgkin's lymphoma, POEMS syndrome / osteosclerotic myeloma, primary effusion lymphoma, splenic marginal zone lymphoma, small lymphocytic lymphoma, smoldering multiple myeloma (SMM), and Waldenstrom's macroglobulinemia.

[0153] In particular embodiments, the recipient is receiving genetically-modified hematopoietic stem cells that provide a gene the recipient is lacking. These recipients may have a primary or

secondary immunodeficiency that can be treated with the provision of a therapeutic gene through hematopoietic stem cells. More than 80 primary immune deficiency diseases are recognized by the World Health Organization. These diseases are characterized by an intrinsic defect in the immune system in which, in some cases, the body is unable to produce any or enough antibodies against infection. In other cases, cellular defenses to fight infection fail to work properly. Typically, primary immune deficiencies are inherited disorders. X-linked severe combined immunodeficiency (SCID-X1) is another example of a primary immune deficiency. X-linked SCID results in both a cellular and humoral immune depletion caused by mutations in the common gamma chain gene (γ C), which result in the absence of T and natural killer (NK) lymphocytes.

[0154] Secondary, or acquired, immune deficiencies are not the result of inherited genetic abnormalities, but rather occur in individuals in which the immune system is compromised by factors outside the immune system. Examples include trauma, viruses, chemotherapy, toxins, and pollution. Acquired immunodeficiency syndrome (AIDS) is an example of a secondary immune deficiency disorder caused by a virus, the human immunodeficiency virus (HIV), in which a depletion of T lymphocytes renders the body unable to fight infection.

[0155] In particular embodiments, B cells may be obtained, a subset of the B cells may be modified ex vivo, and then the modified B cells may be formulated and administered to a subject. In particular embodiments, a first set of B cells may be modified with first nucleic acids to produce a selected antibody against an inflammatory molecule, such as an inflammatory cytokine, thereby providing antibodies that protect against inflammation. In particular embodiments, the selected antibodies can be anti-TNF antibodies and/or anti-IL-1 antibodies. In particular embodiments, the selected antibody is infliximab, adalimumab, and/or golimumab and/or an approved biosimilar thereof.

[0156] As indicated, in particular embodiments, modified B cells express a tag that allows, for example, tracking and/or elimination after administration to a subject.

[0157] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from in vitro assays and/or animal model studies. Such information can be used to more accurately determine useful doses in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician, veterinarian or researcher taking into account parameters such as physical and physiological factors including age, previous vaccinations (if any), target, body weight, severity of condition, type of condition, stage of condition, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

[0158] Exemplary doses can include greater than 10^2 modified B cells, greater than 10^3 modified

B cells, greater than 10^4 modified B cells, greater than 10^5 modified B cells, greater than 10^6 modified B cells, greater than 10^7 modified B cells, greater than 10^8 modified B cells, greater than 10^9 modified B cells, greater than 10^{10} modified B cells, or greater than 10^{11} modified B cells.

[0159] In particular embodiments, the effects of selected antibodies can be measured using viral titers. Viral titer refers to the amount of virus that can be detected. High viral titers mean high levels of infection. An optimal protective response is observed with titers that fall to zero.

[0160] The Exemplary Embodiments and Example(s) below are included to demonstrate particular embodiments of the disclosure. Those of ordinary skill in the art should recognize in light of the present disclosure that many changes can be made to the specific embodiments disclosed herein and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

[0161] (xii) Exemplary Embodiments.

1. A fusion protein, the expression of which in a B cell, results in CD40 activation independently of CD40 ligand (CD40L) binding.
2. The fusion protein of embodiment 1, including an antigen binding domain linked to a transmembrane domain that is linked to an intracellular CD40 signaling domain.
3. The fusion protein of embodiment 2, wherein the antigen binding domain is an engineered antigen binding domain.
4. The fusion protein of embodiment 3, wherein the engineered antigen binding domain is a single chain variable fragment (scFv).
5. The fusion protein of embodiments 3 and 4, wherein the engineered antigen binding domain is linked to the transmembrane domain through a spacer.
6. The fusion protein of embodiment 5, wherein the spacer is an extracellular portion of CD40 or an IgG hinge region.
7. The fusion protein of any of embodiments 2-6, further including a tag.
8. The fusion protein of embodiment 7, wherein the tag has a sequence set forth in SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, or SEQ ID NO: 63.
9. The fusion protein of any of embodiments 2-8, further including at least two tags including (i) SEQ ID NO: 62 or SEQ ID NO: 50 and (ii) SEQ ID NO: 51.
10. The fusion protein of embodiments 7 and 8, wherein the tag links the antigen binding domain to the spacer.
11. The fusion protein of any of embodiments 3-10, wherein the engineered antigen binding

domain is derived from the binding domain of an anti-Respiratory Syncytial Virus (RSV) antibody, an anti-human immunodeficiency virus (HIV) antibody, an anti-Dengue virus antibody, an anti-Bordetella pertussis antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-Clostridium difficile bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.

12. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-RSV antibody including a heavy chain including the sequence set forth in SEQ ID NO: 78 and a light chain including the sequence set forth in SEQ ID NO: 79; or an anti-RSV antibody including a heavy chain including the sequence set forth in SEQ ID NO: 80 and a light chain including the sequence set forth in SEQ ID NO: 81.

13. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-RSV antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 82, a CDRH2 including the sequence set forth in SEQ ID NO: 83, a CDRH3 including the sequence set forth in SEQ ID NO: 84; a CDRL1 including the sequence set forth in SEQ ID NO: 85, a CDRL2 including the sequence set forth in SEQ ID NO: 86, and a CDRL3 including the sequence set forth in SEQ ID NO: 87.

14. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HIV antibody including 10E8, VRC01, ab18633 or 39/5.4A.

15. The fusion protein of any of embodiments 3-11 and 14, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HIV antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 88, a CDRH2 including the sequence set forth in SEQ ID NO: 89, a CDRH3 including the sequence set forth in SEQ ID NO: 90, a CDRL1 including the sequence set forth in SEQ ID NO: 91, a CDRL2 including the sequence set forth in SEQ ID NO: 92, and a CDRL3 including the sequence set forth in SEQ ID NO: 93 or a CDRH1 including the sequence set forth in SEQ ID NO: 94, a CDRH2 including the sequence set forth in SEQ ID NO: 95, a CDRH3 including the sequence set forth in SEQ ID NO: 96, a CDRL1 including the sequence set forth in SEQ ID NO: 97, a CDRL2 including the sequence SGS, and a CDRL3 including the sequence set forth in SEQ ID NO: 98.

16. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Dengue virus antibody including antibody 55, DB2-3, ab155042 or ab80914.

17. The fusion protein of any of embodiments 3-11 and 16, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Dengue virus antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 99, a CDRH2 including the sequence set forth in SEQ ID NO: 100, a CDRH3 including the sequence set forth in SEQ ID NO: 101; a CDRL1 including the sequence set forth in SEQ ID NO: 102, a CDRL2 including the sequence set forth in SEQ ID NO: 103, and a CDRKL3 including the sequence set forth in SEQ ID NO: 104 or a CDRH1 including the sequence set forth in SEQ ID NO: 105, a CDRH2 including the sequence set forth in SEQ ID NO: 106, a CDRH3 including the sequence set forth in SEQ ID NO: 107, a CDRL1 including the sequence set forth in SEQ ID NO: 108, a CDRL2 including the sequence set forth in SEQ ID NO: 109, and a CDRL3 including the sequence set forth in SEQ ID NO: 110.
18. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-pertussis antibody including a heavy chain including the sequence set forth in SEQ ID NO: 111 and a light chain including the sequence set forth in SEQ ID NO: 112.
19. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-hepatitis C antibody including MAB8694 or C7-50.
20. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-hepatitis C antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 113, a CDRH2 including the sequence set forth in SEQ ID NO: 114, a CDRH3 including the sequence set forth in SEQ ID NO: 115, a CDRL1 including the sequence set forth in SEQ ID NO: 116, a CDRL2 including the sequence set forth in SEQ ID NO: 117, and a CDRL3 including the sequence set forth in SEQ ID NO: 118.
21. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-influenza virus antibody including C102.
22. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-influenza virus antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 119, a CDRH2 including the sequence set forth in SEQ ID NO: 120, a CDRH3 including the sequence set forth in SEQ ID NO: 121, a CDRL1 including the sequence set forth in SEQ ID NO: 122, a CDRL2 including the sequence KTS, and a CDRL3 including the sequence set forth in SEQ ID NO: 123.
23. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-MPV antibody including MPE8.
24. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding

domain is derived from the binding domain of an anti-CMV antibody including MCMV5322A, MCMV3068A, LJP538, or LJP539.

25. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-EBV antibody including a CDRH1 including the sequence set forth in SEQ ID NO: 124, a CDRH2 including the sequence set forth in SEQ ID NO: 125, a CDRH3 including the sequence set forth in SEQ ID NO: 126, a CDRL1 including the sequence set forth in SEQ ID NO: 127, a CDRL2 including the sequence set forth in SEQ ID NO: 128, and a CDRL3 including the sequence set forth in SEQ ID NO: 129.

26. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HSV antibody including HSV8-N and MB66.

27. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Clostridium difficile antibody including actoxumab or bezlotoxumab.

28. The fusion protein of any of embodiments 3-11, wherein the engineered antigen binding domain is derived from the binding domain of an anti-TNF antibody including infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.

29. The fusion protein of any of embodiments 4-28, wherein the scFv includes a Gly-Ser linker having 5-30 amino acids.

30. The fusion protein of embodiment 29, wherein the Gly-Ser linker has 15 amino acids.

31. The fusion protein of any of embodiments 2-30, further including a Gly linker.

32. The fusion protein of embodiment 31, wherein the Gly linker is Gly₆.

33. The fusion protein of embodiments 31 and 32, wherein the Gly linker links a tag to a spacer region or a tag to an intracellular CD40 signaling domain.

34. The fusion protein of any of embodiments 2-33, having a sequence set forth in SEQ ID NO: 4, 6, 8, or 10.

35. The fusion protein of any of embodiments 1-34, including CD79 α linked to an intracellular CD40 signaling domain or CD79 β linked to an intracellular CD40 signaling domain.

36. The fusion protein of any of embodiments 2-35, further including a multimerization domain.

37. The fusion protein of embodiment 36, wherein the multimerization domain includes FKBP12 or FKBP12v36.

38. The fusion protein of any of embodiments 2-37, having a sequence set forth in SEQ ID NO: 14, 16, 18, or 20.

39. A B cell expressing the fusion protein of any of embodiments 2-38.

40. A nucleic acid encoding the fusion protein of any of embodiments 2-38.

41. The nucleic acid of embodiment 40, wherein the nucleic acid further encodes a signal peptide.
42. The nucleic acid of embodiment 41, wherein the signal peptide is a CD40 signal peptide.
43. The nucleic acid of any of embodiments 40-42, wherein the nucleic acid further encodes a skipping element, and a reporter.
44. The nucleic acid of embodiment 43, wherein the skipping element is a self-cleaving peptide.
45. The nucleic acid of embodiment 44, wherein the self-cleaving peptide is a 2A self-cleaving peptide.
46. The nucleic acid of any of embodiments 43-45, wherein the reporter is a fluorescent protein or truncated epidermal growth factor receptor (tEGFR).
47. The nucleic acid of any of embodiments 40-46, having a sequence set forth in SEQ ID NO: 3, 5, 7, 9, 13, 15, 17, or 19.
48. A B cell including the nucleic acid of any of embodiments 40-47.
49. The B cell of embodiment 48, further including a nuclease and guide RNA (gRNA) that results in deletion of a gene encoding a transcription factor required for MHC class II molecule expression.
50. The B cell of embodiment 49, wherein the transcription factor includes CIITA, TRAC, TRBC, B2M, RFX5, RFXAP, or RFXANK.
51. The B cell of embodiments 49 and 50, wherein the nuclease is Cas9 or Cpf1.
52. The B cell of any of embodiments 49-51, wherein the gRNA has the sequence set forth in SEQ ID NO: 1, 2, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, or 202.
53. The B cell of any of embodiments 39 and 48-52, wherein the B cell is an antibody-secreting B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.
54. The B cell of any of embodiments 48-53, formulated for administration to a subject.
55. A kit including a nucleic acid encoding the fusion protein of any of embodiments 2-38.
56. The kit of embodiment 55, further including a nucleic acid vector.
57. The kit of embodiment 56, wherein the nucleic acid vector includes a plasmid, a transposon, a cosmid, or a viral vector.
58. The kit of any of embodiments 55-57, further including a nuclease.
59. The kit of embodiment 58, wherein the nuclease is Cas9 or Cpf1.
60. The kit of any of embodiments 55-59, further including guideRNA (gRNA).
61. The kit of embodiment 60, wherein the gRNA has a sequence set forth in SEQ ID NO: 1, 2,

134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, or 202.

62. The kit of any of embodiments 58-61, wherein the gRNA and nuclease are associated with a nanoparticle.

63. A method of genetically modifying B cells to express a fusion protein that allows CD40 activation of the B cell based on antigen binding and independently of CD40L binding wherein the method includes introducing the nucleic acid of any of embodiments 40 -47 into the B cell.

64. The method of embodiment 63, further including genetically modifying the B cell to delete expression of a transcription factor required for MHC class II molecule expression.

65. The method of embodiment 64, wherein the transcription factor includes CIITA, TRAC, TRBC, B2M, RFX5, or RFXAP.

66. A method of providing an anti-infection effect in a subject in need thereof including administering a therapeutically effective amount of the B cell of any of embodiments 39 and 48-54 to the subject thereby providing an anti-infection effect.

67. The method of embodiment 66, wherein the providing obviates the need for a vaccination.

68. The method of embodiments 66 and 67, wherein the administering replaces a vaccination protocol.

69. The method of any of embodiments 66-68, wherein the subject is immune-suppressed.

70. The method of any of embodiments 66-69, wherein the subject is immune-suppressed as part of a treatment regimen including a bone marrow transplant, hematopoietic stem cell transplant, or administration of genetically modified hematopoietic stem cells.

71. A method of providing an anti-inflammatory effect in a subject in need thereof including administering a therapeutically effective amount of a B cell of any of embodiments 39 and 48-54 to the subject thereby providing an anti-inflammatory effect.

[0162] (xiii) Experimental Examples. Example 1. scFv CD40. The following constructs were generated using Gibson cloning:

- Construct 1: humanCD40 signal peptide - anti-gp120 scFv - Linker - StrepTag - Gly6 Linker- full CD40 protein - Gly6 Linker - HA tag - P2A skipping element - mCherry reporter;
- Construct 2: humanCD40 signal peptide - anti-gp120 scFv - Linker - StrepTagII- Gly6 Linker- IgG Hinge- Gly6 Linker- CD40 Transmembrane Domain - CD40 Intracellular Domain - Gly6 Linker - HA tag - P2A skipping element - mCherry reporter;
- Construct 3: humanCD40 signal peptide - anti-gp120 scFv - Linker - StrepTagII- Gly6

Linker- IgG Hinge- Gly6 Linker- IgG2 Transmembrane Domain - Gly6 Linker - CD40 Intracellular Domain - Gly6 Linker - HA tag - P2A skipping element - mCherry reporter;

- Construct 4: humanCD40 signal peptide - anti-gp120 scFv - Linker - StrepTag - Gly6 Linker- full CD40 protein - Gly6 Linker- FkBP12v36 - GlySer Linker - FkBP12v36- Gly6 Linker - HA tag - P2A skipping element - mCherry reporter; and
- Construct 5: human CD40 signal peptide - full CD40 protein - Gly6 Linker - HA tag - P2A skipping element - mCherry Reporter.

The constructs were amplified in E. Coli and the DNA was isolated by Qiagen Midi Prep kit. The DNA was then chemically transfected into HEK293E cells. After 36 hours incubation, the cells were harvested and stained with the following panel for flow cytometry analysis of expression.

Antibody	Color	Function
p-p38	PE-Cy7	Phosphorylated downstream of CD40 signaling
p-ERK	AF700	Phosphorylated downstream of CD40 signaling
p-Stat3	488	Phosphorylated downstream of CD40 signaling
	mCherry	Plasmid in cell
SA	BV421	Extracellular tag on construct
HA	AF647	Intracellular tag on construct
Ghost Dye	V510	Live/dead

For functional testing, Ramos cells were electroporated via Neon protocol for the conditions outlined below:

1. Construct 1 stimulated w/ HIV Env tetramer;
2. Construct 1 stimulated w/ Streptactin-PE;
3. Construct 1 no stim;
4. Construct 2 stimulated w/ HIV Env tetramer;
5. Construct 2 stimulated w/ Streptactin-PE;
6. Construct 2 no stim;
7. Construct 3 stimulated w/ HIV Env tetramer;
8. Construct 3 stimulated w/ Streptactin-PE;
9. Construct 3 no stim;
10. Construct 4 stimulated w/ AP1903; and
11. Construct 4 no stim

After 24 hour incubation, the cells were harvested and stained for flow cytometry experiments.

[0163] Example 2. BCR-CD40 Complexes. The following constructs were created using Gibson

cloning:

- MRT3: mouseCD79 α -IntracellularCD40-P2A skipping element -eGFP Reporter;
- MRT4: mouseCD79 β -IntracellularCD40-P2A skipping element -eGFP Reporter;
- MRT5: humanCD79 α -IntracellularCD40-P2A skipping element -mCherry Reporter;
- MRT6: humanCD79 β -IntracellularCD40-P2A skipping element -mCherry Reporter;
- MRT7: humanCD79 α -P2A skipping element -mCherry Reporter; and
- MRT8: humanCD79 β -P2A skipping element -mCherryReporter

These constructs were amplified in E. Coli and the DNA was isolated by Qiagen Midi Prep kit. The DNA was then chemically transfected into HEK293E cells for the conditions outlined below:

[0164] Conditions:

- WT CD79 α + WT CD79 β + WT anti-huRSV;
- Mutated CD79 α + WT CD79 β + WT anti-huRSV;
- WT CD79 α + Mutated CD79 β + WT anti-huRSV; and
- Mutated CD79 α + Mutated CD79 β + WT anti-huRSV

[0165] After 36 hours incubation, the cells were harvested and stained with the following panel for flow cytometry analysis of expression.

Item	Fluorescence	Channel	Marker
monomer RSV tag	Blue	B510	Fitc, AF488
mCherry	Green	G610	PE-eF610
FVD	Violet	V510	Ghost 510
IgD	UV	U737	BUV 737
anti huCD79 β	Red	R780	APC Fire 750

[0166] For functional testing, Ramos cells were electroporated via Neon protocol for the following conditions (mock and sample 1-4 for each below):

- IgG stimulation, 24 hours/24+ hours incubation, phospho-flow;
- RSV-preF stimulation, 24 hours/24+ hours incubation, phospho-flow;
- No stimulation, 48 total hours incubation, phospho-flow;
- No stimulation, 24 total hours incubation, Calcium Flux; and
- No stimulation, 48 total hours incubation, Calcium Flux

After 24-hour incubation, the cells were harvested and stained for flow cytometry experiments via the following panel:

Intra-cellular Stain		
Channel	Fluor	Target
B510	AF488	Stat3
R660	AF647	CD79 α
R710	AF700	ERK1/2
G575	PE	Syk
G610		mCherry
G780	PE-Cy7	P38
	BV Buffer	
Extracellular Stain		
R780	APC Fire 780	antiCD79 β
U737	BUV 737	IgD
V510	Ghost508	Live/Dead

[0167] (xiv) Closing Paragraphs. Variants of protein and nucleic acid sequences described herein are also included. Variants of proteins can include those having one or more conservative amino acid substitutions or one or more non-conservative substitutions that do not adversely affect the binding or activity of the protein.

[0168] In particular embodiments, a conservative amino acid substitution may not substantially change the structural characteristics of the reference sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the reference sequence or disrupt other types of secondary structure that characterizes the reference sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden & J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., *Nature*, 354:105 (1991).

[0169] In particular embodiments, a “conservative substitution” involves a substitution found in one of the following conservative substitutions groups: Group 1: Alanine (Ala), Glycine (Gly), Serine (Ser), Threonine (Thr); Group 2: Aspartic acid (Asp), Glutamic acid (Glu); Group 3: Asparagine (Asn), Glutamine (Gln); Group 4: Arginine (Arg), Lysine (Lys), Histidine (His); Group 5: Isoleucine (Ile), Leucine (Leu), Methionine (Met), Valine (Val); and Group 6: Phenylalanine (Phe), Tyrosine (Tyr), Tryptophan (Trp).

[0170] Additionally, amino acids can be grouped into conservative substitution groups by similar function or chemical structure or composition (e.g., acidic, basic, aliphatic, aromatic, sulfur-

containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other groups containing amino acids that are considered conservative substitutions for one another include: sulfur-containing: Met and Cysteine (Cys); acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. Additional information is found in Creighton (1984) *Proteins*, W.H. Freeman and Company.

[0171] In the fusion proteins disclosed herein, amino acid substitutions, deletions or additions can be made in linker sequences and spacer regions. They may also be made within CD40 signaling domains so long as the substitution, deletion, or addition does not adversely affect CD40 activation signals.

[0172] In particular embodiments, a V_L region can be derived from or based on a disclosed V_L and can include one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the disclosed V_L . An insertion, deletion or substitution may be anywhere in the V_L region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided an antibody including the modified V_L region can still specifically bind its target epitope with an affinity similar to the wild type binding domain.

[0173] In particular embodiments, a V_H region can be derived from or based on a disclosed V_H and can include one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) insertions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) deletions, one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions (e.g., conservative amino acid substitutions or non-conservative amino acid substitutions), or a combination of the above-noted changes, when compared with the V_H disclosed herein. An insertion, deletion or substitution may be anywhere in the V_H region, including at the amino- or carboxy-terminus or both ends of this region, provided that each CDR includes zero changes or at most one, two, or three changes and provided an antibody including the modified V_H region can still specifically bind its target epitope with an affinity similar to the wild type binding domain.

[0174] In particular embodiments, a variant includes or is a sequence that has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% sequence identity to a sequence disclosed herein. In particular embodiments, a variant includes or is a sequence that has at least 90%, at least 91%,

at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% sequence identity to a light chain variable region (V_L) and/or to a heavy chain variable region (V_H), or both, wherein each CDR includes zero changes or at most one, two, or three changes, from the reference antibody disclosed herein or fragment or derivative thereof that binds the relevant antigen.

[0175] Each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms “include” or “including” should be interpreted to recite: “comprise, consist of, or consist essentially of.” The transition term “comprise” or “comprises” means has, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase “consisting of” excludes any element, step, ingredient or component not specified. The transition phrase “consisting essentially of” limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant reduction in CD40 signaling following antigen binding, independently of interactions with CD40L through T cell interaction.

[0176] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term “about” has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of $\pm 20\%$ of the stated value; $\pm 19\%$ of the stated value; $\pm 18\%$ of the stated value; $\pm 17\%$ of the stated value; $\pm 16\%$ of the stated value; $\pm 15\%$ of the stated value; $\pm 14\%$ of the stated value; $\pm 13\%$ of the stated value; $\pm 12\%$ of the stated value; $\pm 11\%$ of the stated value; $\pm 10\%$ of the stated value; $\pm 9\%$ of the stated value; $\pm 8\%$ of the stated value; $\pm 7\%$ of the stated value; $\pm 6\%$ of the stated value; $\pm 5\%$ of the stated value; $\pm 4\%$ of the stated value; $\pm 3\%$ of the stated value; $\pm 2\%$ of the stated value; or $\pm 1\%$ of the stated value.

[0177] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are

reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0178] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0179] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0180] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0181] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety

for their referenced teaching.

[0182] It is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0183] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0184] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the example(s) or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 11th Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of Biochemistry and Molecular Biology, 2nd Edition (Ed. Anthony Smith, Oxford University Press, Oxford, 2006) or Janeway's Immunobiology, 9th Edition (Murphy & Weaver, Garland Science, 2017).

CLAIMS

What is claimed is:

1. A fusion protein, the expression of which in a B cell, results in CD40 activation independently of CD40 ligand (CD40L) binding.
2. The fusion protein of claim 1, comprising an antigen binding domain linked to a transmembrane domain that is linked to an intracellular CD40 signaling domain.
3. The fusion protein of claim 2, wherein the antigen binding domain is an engineered antigen binding domain.
4. The fusion protein of claim 3, wherein the engineered antigen binding domain is a single chain variable fragment (scFv).
5. The fusion protein of claim 3, wherein the engineered antigen binding domain is linked to the transmembrane domain through a spacer.
6. The fusion protein of claim 5, wherein the spacer is an extracellular portion of CD40 or an IgG hinge region.
7. The fusion protein of claim 2, further comprising a tag.
8. The fusion protein of claim 7, wherein the tag has a sequence set forth in SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, or SEQ ID NO: 63.
9. The fusion protein of claim 2, further comprising at least two tags comprising (i) SEQ ID NO: 62 or SEQ ID NO: 50 and (ii) SEQ ID NO: 51.
10. The fusion protein of claim 7, wherein the tag links the antigen binding domain to the spacer.
11. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Respiratory Syncytial Virus (RSV) antibody, an anti-human immunodeficiency virus (HIV) antibody, an anti-Dengue virus antibody, an anti-Bordetella pertussis antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-Clostridium difficile bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.
12. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-RSV antibody comprising a heavy chain comprising the sequence set forth in SEQ ID NO: 78 and a light chain comprising the sequence set forth in SEQ ID NO: 79; or an anti-RSV antibody comprising a heavy chain comprising the sequence set forth in SEQ ID

NO: 80 and a light chain comprising the sequence set forth in SEQ ID NO: 81.

13. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-RSV antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 82, a CDRH2 comprising the sequence set forth in SEQ ID NO: 83, a CDRH3 comprising the sequence set forth in SEQ ID NO: 84; a CDRL1 comprising the sequence set forth in SEQ ID NO: 85, a CDRL2 comprising the sequence set forth in SEQ ID NO: 86, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 87.

14. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HIV antibody comprising 10E8, VRC01, ab18633 or 39/5.4A.

15. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HIV antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 88, a CDRH2 comprising the sequence set forth in SEQ ID NO: 89, a CDRH3 comprising the sequence set forth in SEQ ID NO: 90, a CDRL1 comprising the sequence set forth in SEQ ID NO: 91, a CDRL2 comprising the sequence set forth in SEQ ID NO: 92, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 93 or a CDRH1 comprising the sequence set forth in SEQ ID NO: 94, a CDRH2 comprising the sequence set forth in SEQ ID NO: 95, a CDRH3 comprising the sequence set forth in SEQ ID NO: 96, a CDRL1 comprising the sequence set forth in SEQ ID NO: 97, a CDRL2 comprising the sequence SGS, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 98.

16. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Dengue virus antibody comprising antibody 55, DB2-3, ab155042 or ab80914.

17. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Dengue virus antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 99, a CDRH2 comprising the sequence set forth in SEQ ID NO: 100, a CDRH3 comprising the sequence set forth in SEQ ID NO: 101; a CDRL1 comprising the sequence set forth in SEQ ID NO: 102, a CDRL2 comprising the sequence set forth in SEQ ID NO: 103, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 104 or a CDRH1 comprising the sequence set forth in SEQ ID NO: 105, a CDRH2 comprising the sequence set forth in SEQ ID NO: 106, a CDRH3 comprising the sequence set forth in SEQ ID NO: 107, a CDRL1 comprising the sequence set forth in SEQ ID NO: 108, a CDRL2 comprising the sequence set forth in SEQ ID NO: 109, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 110.

18. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-pertussis antibody comprising a heavy chain comprising the

sequence set forth in SEQ ID NO: 111 and a light chain comprising the sequence set forth in SEQ ID NO: 112.

19. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-hepatitis C antibody comprising MAB8694 or C7-50.

20. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-hepatitis C antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 113, a CDRH2 comprising the sequence set forth in SEQ ID NO: 114, a CDRH3 comprising the sequence set forth in SEQ ID NO: 115, a CDRL1 comprising the sequence set forth in SEQ ID NO: 116, a CDRL2 comprising the sequence set forth in SEQ ID NO: 117, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 118.

21. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-influenza virus antibody comprising C102.

22. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-influenza virus antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 119, a CDRH2 comprising the sequence set forth in SEQ ID NO: 120, a CDRH3 comprising the sequence set forth in SEQ ID NO: 121, a CDRL1 comprising the sequence set forth in SEQ ID NO: 122, a CDRL2 comprising the sequence KTS, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 123.

23. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-MPV antibody comprising MPE8.

24. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-CMV antibody comprising MCMV5322A, MCMV3068A, LJP538, or LJP539.

25. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-EBV antibody comprising a CDRH1 comprising the sequence set forth in SEQ ID NO: 124, a CDRH2 comprising the sequence set forth in SEQ ID NO: 125, a CDRH3 comprising the sequence set forth in SEQ ID NO: 126, a CDRL1 comprising the sequence set forth in SEQ ID NO: 127, a CDRL2 comprising the sequence set forth in SEQ ID NO: 128, and a CDRL3 comprising the sequence set forth in SEQ ID NO: 129.

26. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-HSV antibody comprising HSV8-N and MB66.

27. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-Clostridium difficile antibody comprising actoxumab or bezlotoxumab.

28. The fusion protein of claim 3, wherein the engineered antigen binding domain is derived from the binding domain of an anti-TNF antibody comprising infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.
29. The fusion protein of claim 4, wherein the scFv comprises a Gly-Ser linker having 5-30 amino acids.
30. The fusion protein of claim 29, wherein the Gly-Ser linker has 15 amino acids.
31. The fusion protein of claim 2, further comprising a Gly linker.
32. The fusion protein of claim 31, wherein the Gly linker is Gly₆.
33. The fusion protein of claim 31, wherein the Gly linker links a tag to a spacer region or a tag to an intracellular CD40 signaling domain.
34. The fusion protein of claim 2, having a sequence set forth in SEQ ID NO: 4, 6, 8, or 10.
35. The fusion protein of claim 1, comprising CD79 α linked to an intracellular CD40 signaling domain or CD79 β linked to an intracellular CD40 signaling domain.
36. The fusion protein of claim 2 or 35, further comprising a multimerization domain.
37. The fusion protein of claim 36, wherein the multimerization domain comprises FKBP12 or FKBP12v36.
38. The fusion protein of claim 2, having a sequence set forth in SEQ ID NO: 14, 16, 18, or 20.
39. A B cell expressing the fusion protein of claim 2 or 35.
40. A nucleic acid encoding the fusion protein of claim 2 or claim 35.
41. The nucleic acid of claim 40, wherein the nucleic acid further encodes a signal peptide.
42. The nucleic acid of claim 41, wherein the signal peptide is a CD40 signal peptide.
43. The nucleic acid of claim 40, wherein the nucleic acid further encodes a skipping element, and a reporter.
44. The nucleic acid of claim 43, wherein the skipping element is a self-cleaving peptide.
45. The nucleic acid of claim 44, wherein the self-cleaving peptide is a 2A self-cleaving peptide.
46. The nucleic acid of claim 43, wherein the reporter is a fluorescent protein or truncated epidermal growth factor receptor (tEGFR).
47. The nucleic acid of claim 40, having a sequence set forth in SEQ ID NO: 3, 5, 7, 9, 13, 15, 17, or 19.
48. A B cell comprising the nucleic acid of claim 40.
49. The B cell of claim 39 or 48, further comprising a nuclease and guide RNA (gRNA) that results in deletion of a gene encoding a transcription factor required for MHC class II molecule expression.
50. The B cell of claim 49, wherein the transcription factor comprises CIITA, TRAC, TRBC, B2M,

RFX5, RFXAP, or RFXANK.

51. The B cell of claim 49, wherein the nuclease is Cas9 or Cpf1.

52. The B cell of claim 49, wherein the gRNA has the sequence set forth in 1, 2, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, or 202.

53. The B cell of claim 39 or 48, wherein the B cell is an antibody-secreting B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.

54. The B cell of claim 48, formulated for administration to a subject.

55. A kit comprising a nucleic acid encoding the fusion protein of claim 2 and/or claim 35.

56. The kit of claim 55, further comprising a nucleic acid vector.

57. The kit of claim 56, wherein the nucleic acid vector comprises a plasmid, a transposon, a cosmid, or a viral vector.

58. The kit of claim 55, further comprising a nuclease.

59. The kit of claim 58, wherein the nuclease is Cas9 or Cpf1.

60. The kit of claim 55, further comprising guideRNA (gRNA).

61. The kit of claim 60, wherein the gRNA has a sequence set forth in SEQ ID NO: 1, 2, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, or 202.

62. The kit of claims 58 and 60, wherein the gRNA and nuclease are associated with a nanoparticle.

63. A method of genetically modifying B cells to express a fusion protein that allows CD40 activation of the B cell based on antigen binding and independently of CD40L binding wherein the method comprises introducing the nucleic acid of claim 40 into the B cell.

64. The method of claim 63, further comprising genetically modifying the B cell to delete expression of a transcription factor required for MHC class II molecule expression.

65. The method of claim 64, wherein the transcription factor comprises CIITA, TRAC, TRBC, B2M, RFX5, or RFXAP.

66. A method of providing an anti-infection effect in a subject in need thereof comprising administering a therapeutically effective amount of the B cell of claim 39 or 48 to the subject thereby providing an anti-infection effect.

- 67. The method of claim 66, wherein the providing obviates the need for a vaccination.
- 68. The method of claim 66, wherein the administering replaces a vaccination protocol.
- 69. The method of claim 66, wherein the subject is immune-suppressed.
- 70. The method of claim 66, wherein the subject is immune-suppressed as part of a treatment regimen comprising a bone marrow transplant, hematopoietic stem cell transplant, or administration of genetically modified hematopoietic stem cells.
- 71. A method of providing an anti-inflammatory effect in a subject in need thereof comprising administering a therapeutically effective amount of a B cell of claim 39 or 48 to the subject thereby providing an anti-inflammatory effect.

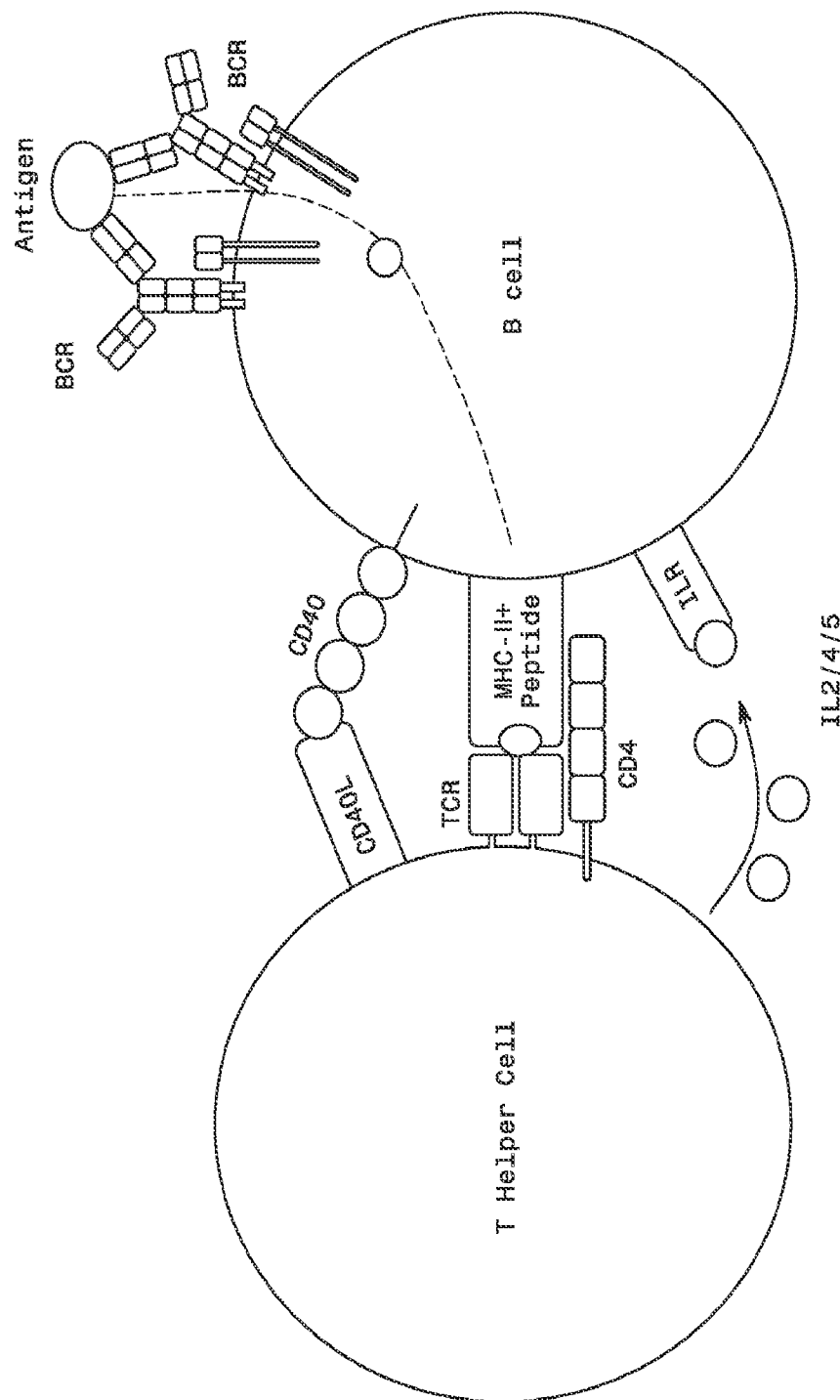


FIG. 1

FIG. 2

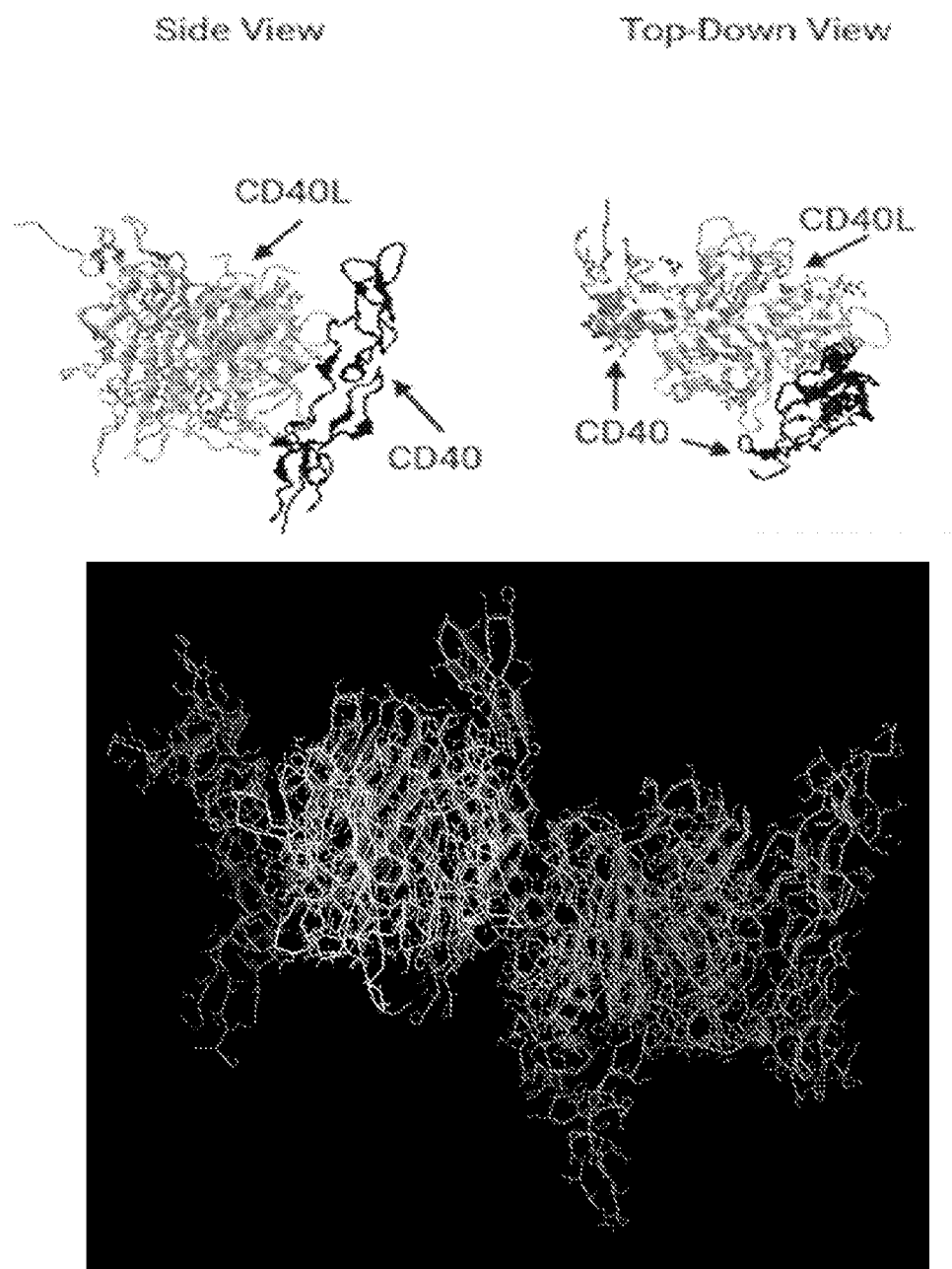
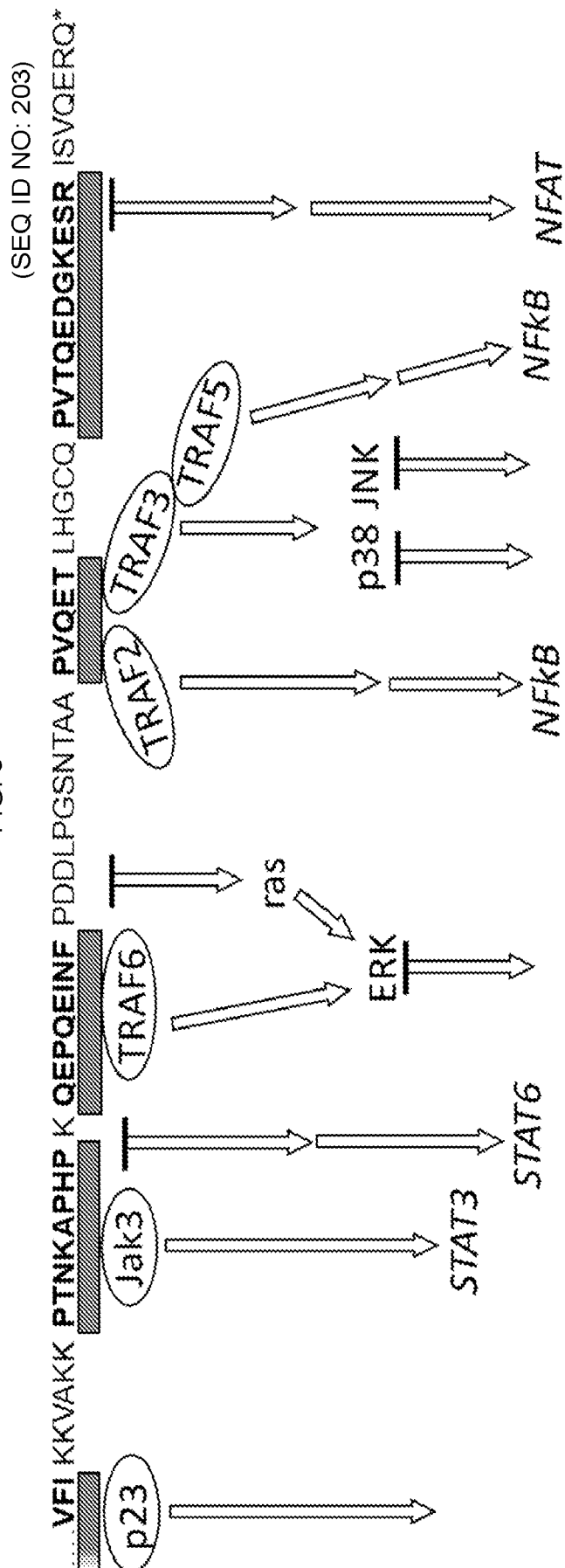


FIG. 3



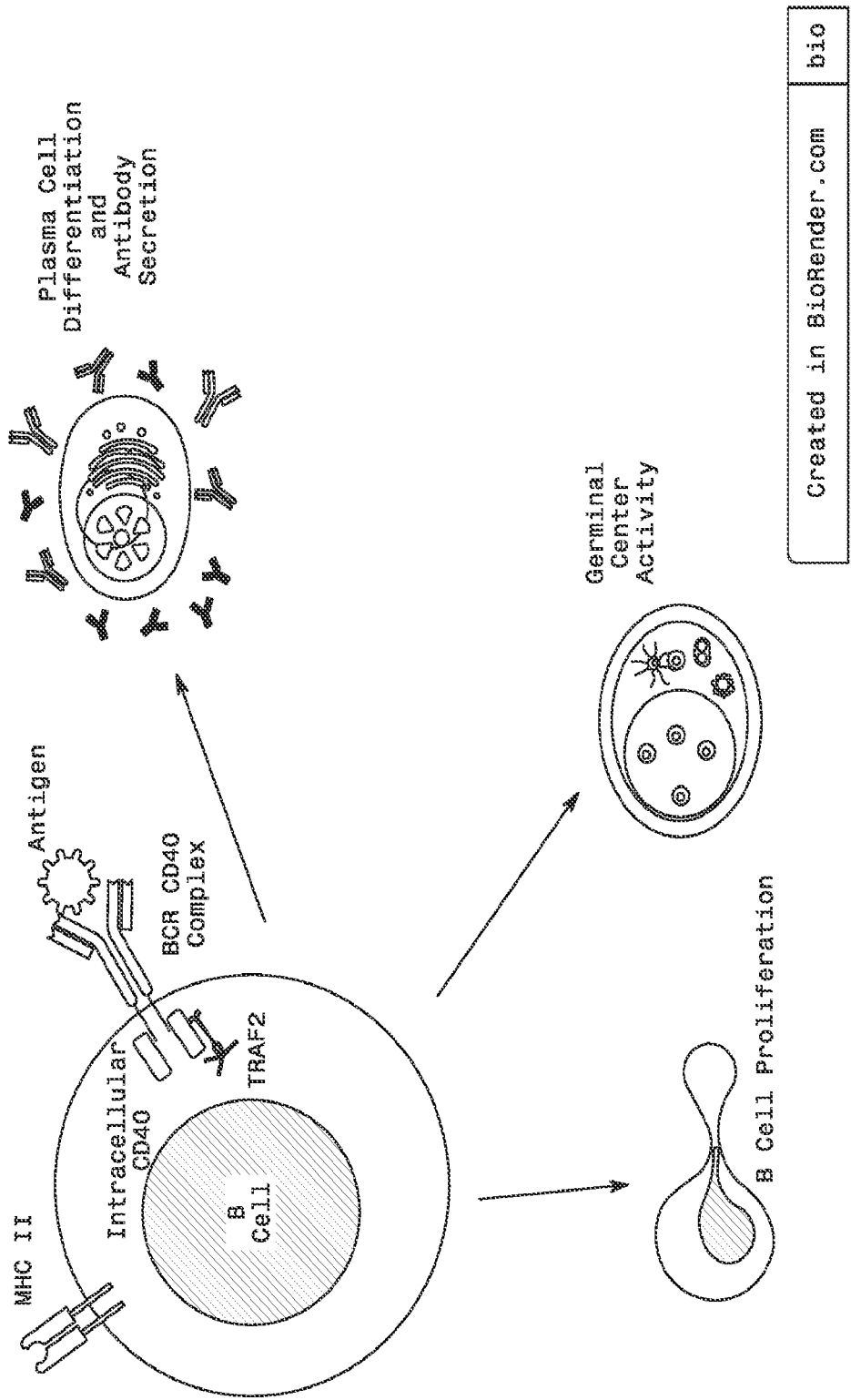


FIG. 4

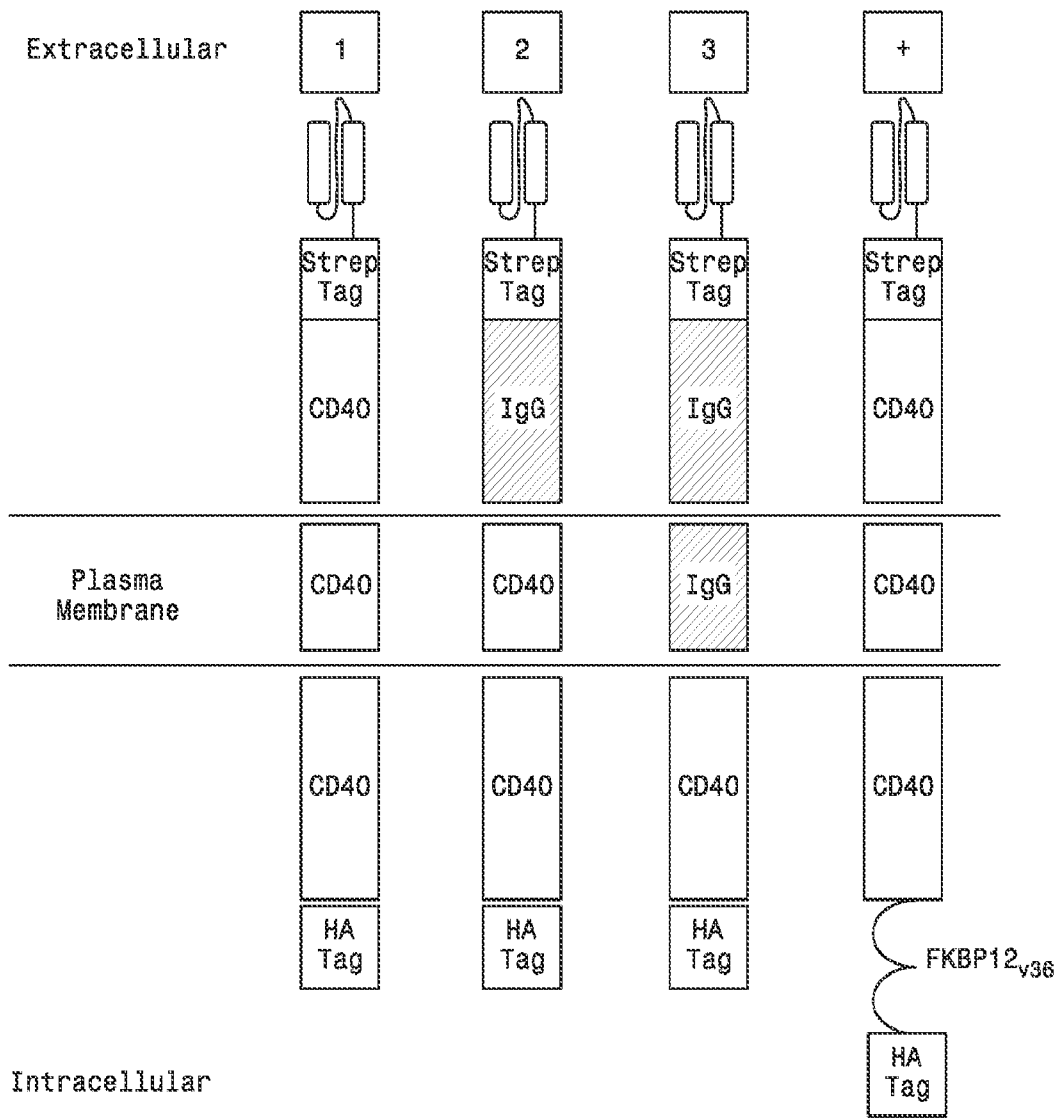


FIG. 5

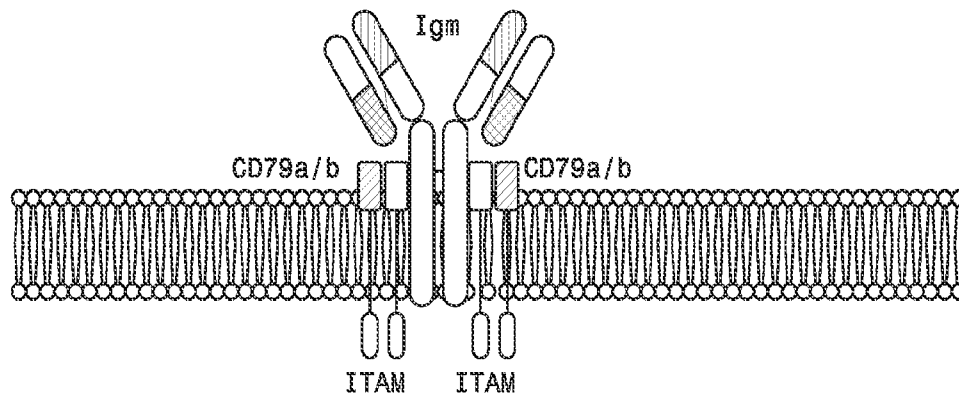


FIG. 6A

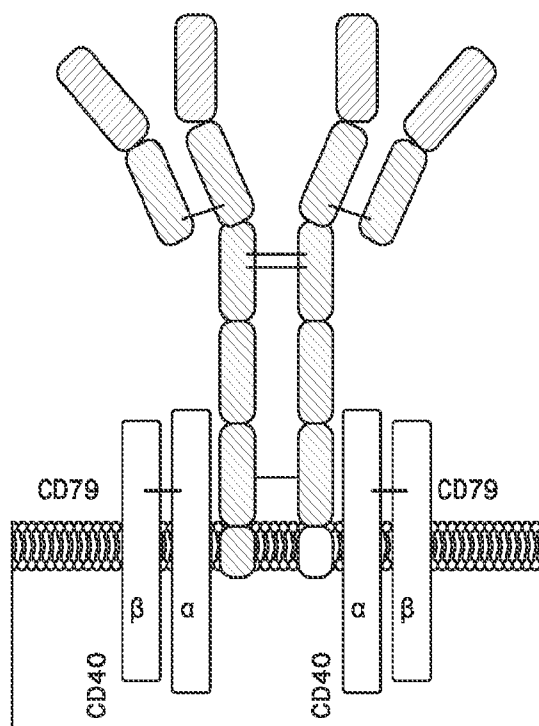


FIG. 6B

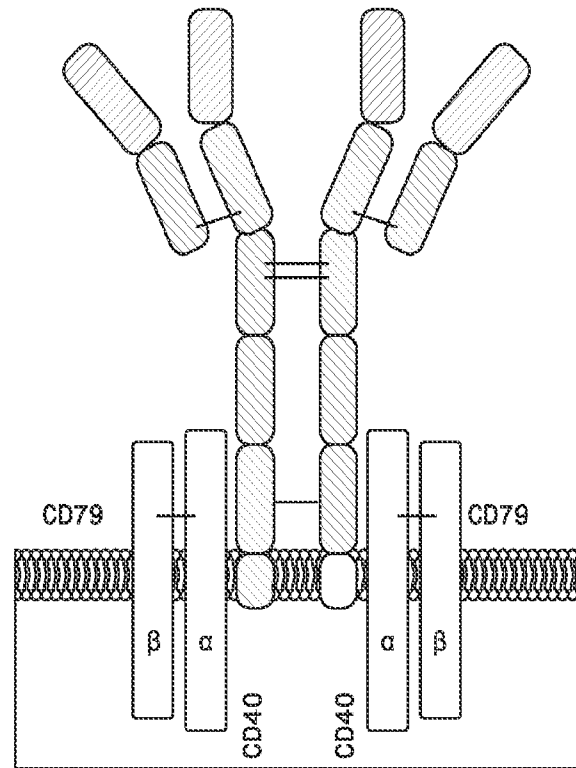


FIG. 6C

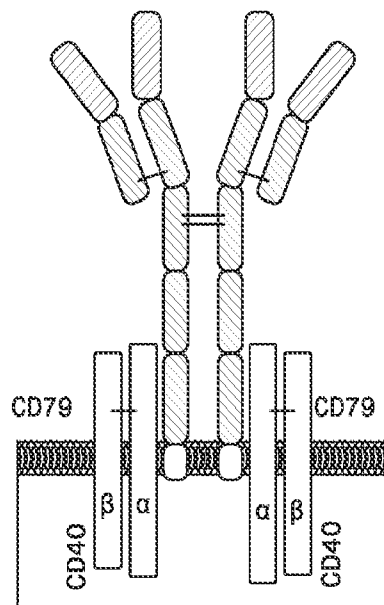
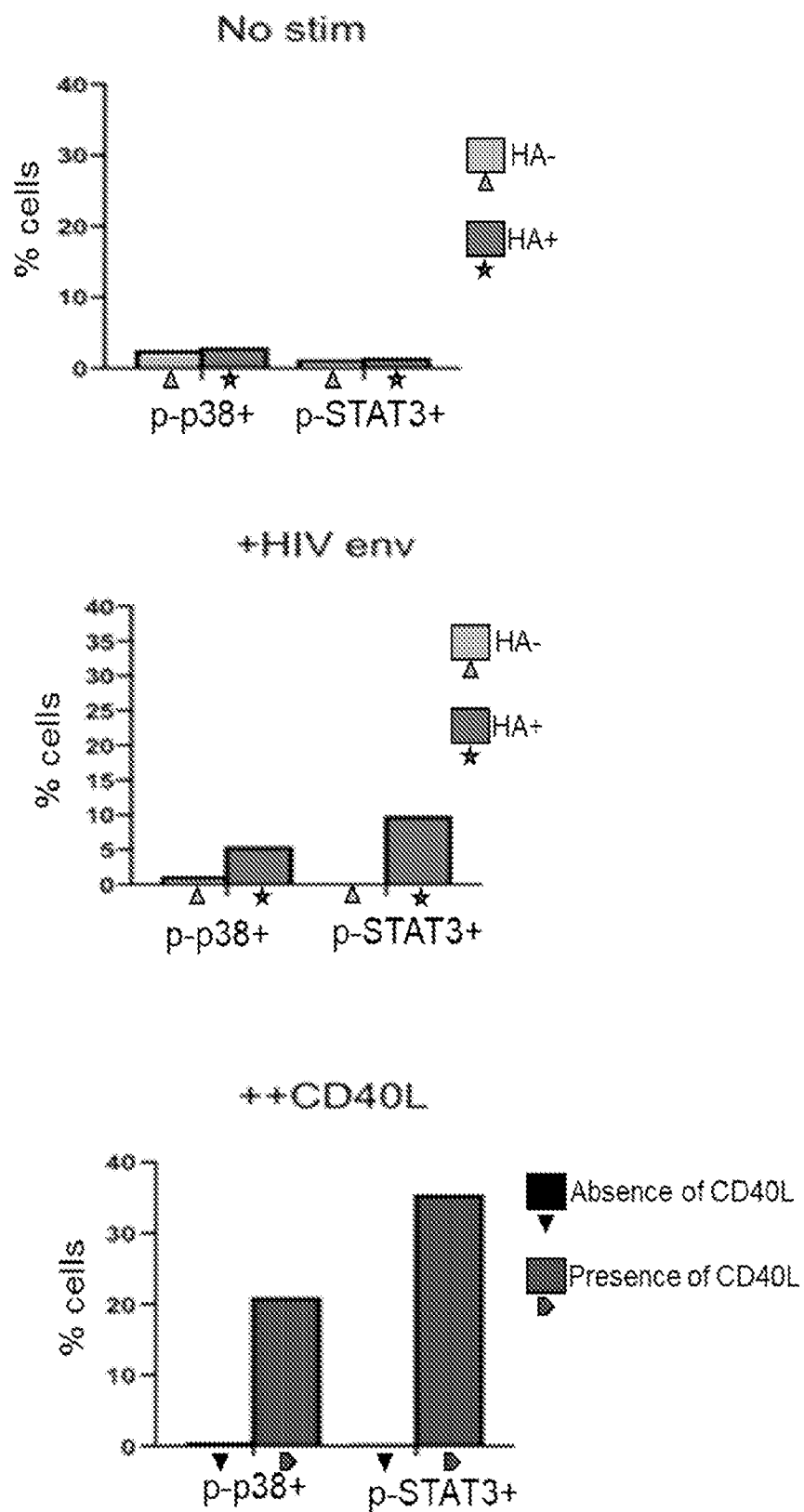


FIG. 6D

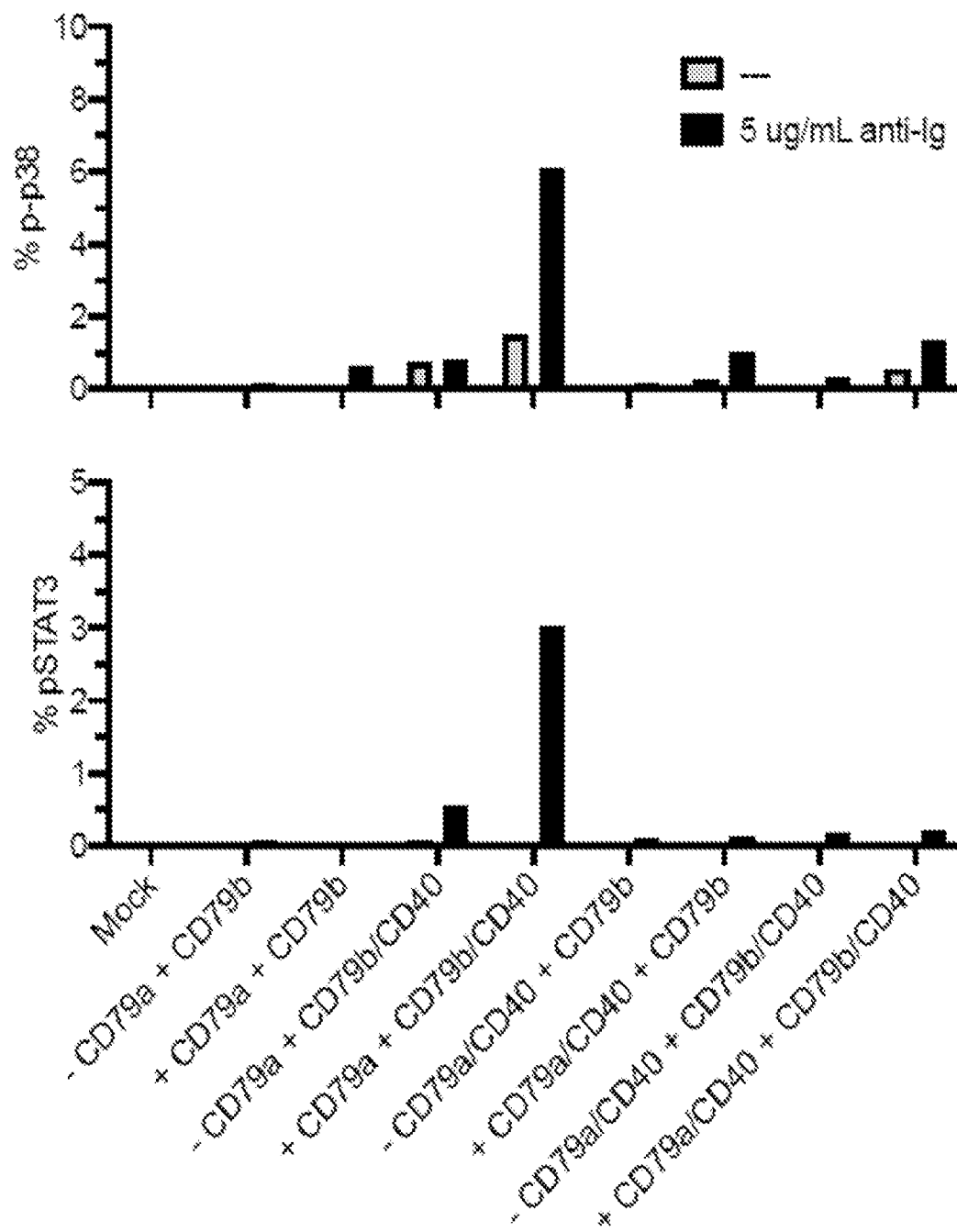
8/150

FIG. 7



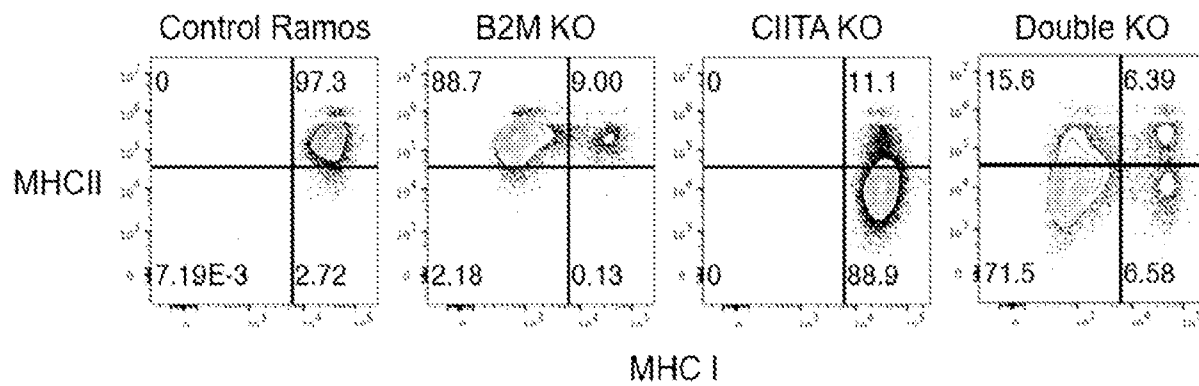
9/150

FIG. 8



10/150

FIG. 9



CIITA sgRNA: GAUAAUUGGCAUAAGCCUCCC (SEQ ID NO: 1)

B2M sgRNA: GGCCGAGAUGUCUCGCUCG (SEQ ID NO: 2)

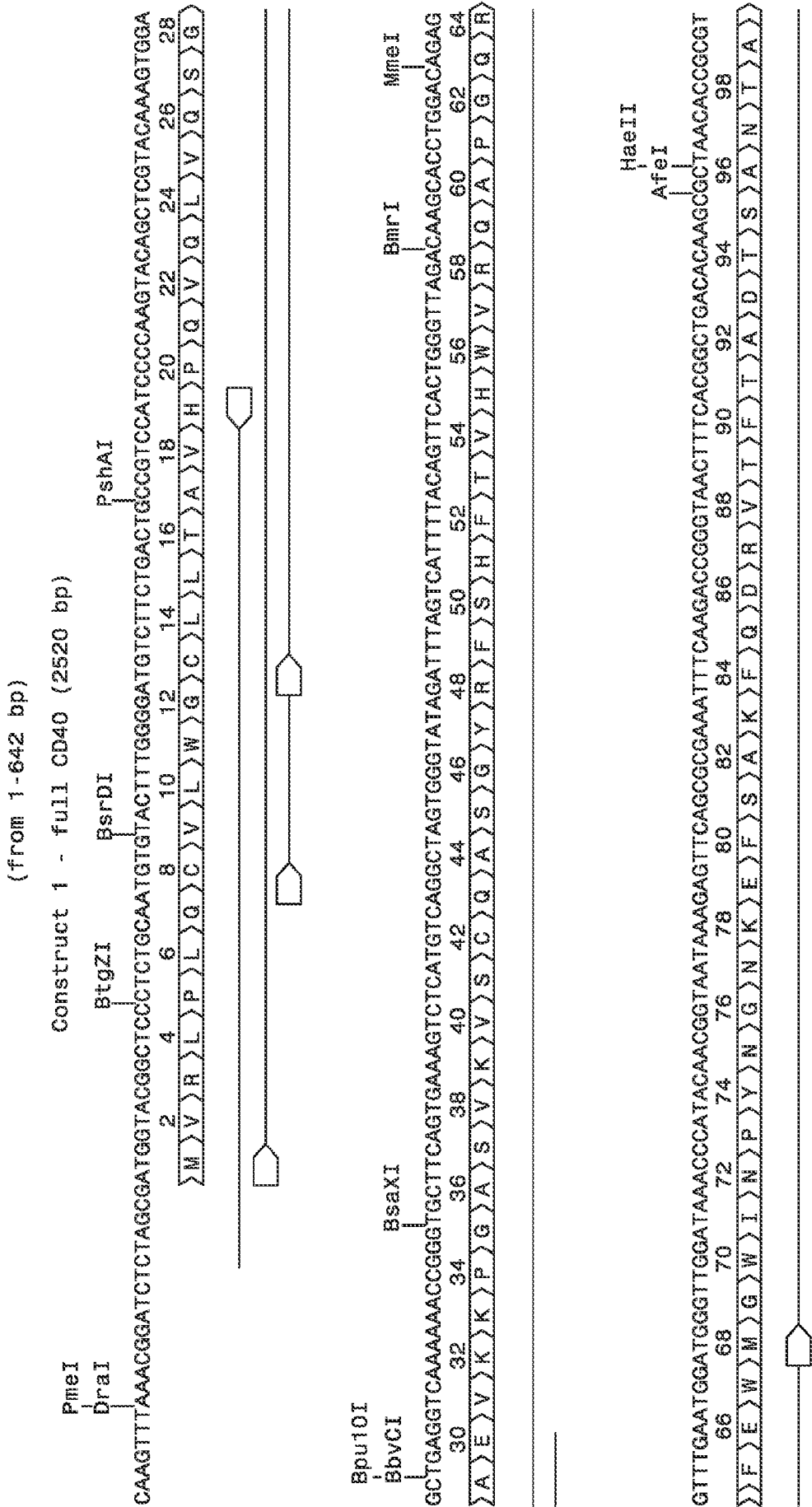


FIG. 10A

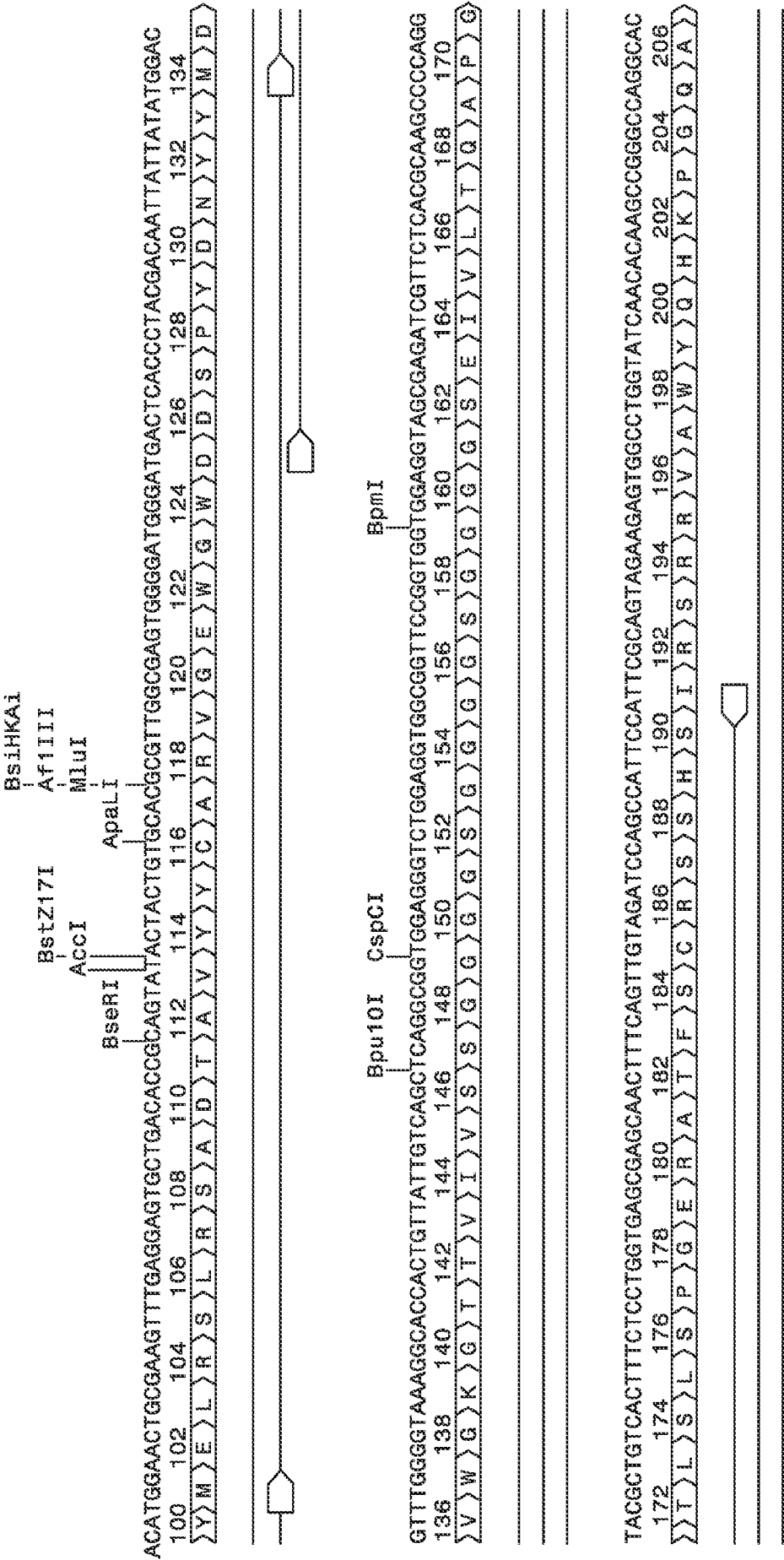


FIG. 10A (Cont'd)

[illegible]

FIG. 10A (Cont'd)

ECORI TffII
BsaAI
GCTCACTGTGCCAGCCGGGTCAGAAAGCTGGTCAGCGACTGTACGGAATTCACTGAAACGGAGTGCCTGCCCTGGGGGAAAGTGAATTCCTTGGATACGTGGAAATCGA
24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58
>C>S>L>C>Q>P>G>Q>K>L>V>S>D>C>T>E>F>T>E>T>E>C>L>P>C>G>E>S>E>F>L>D>T>W>N>R>

ScaI BsmBI BsaXI
GAAACTCATTGTCATCAACACAAGTACTGTGACCCGGAACCTGGGTCTGAGGGTGCAGCAAAAGGCCACCCAGTGAGACGGGATACAAATTTGCACCTGTGAAGAGGGCTG
60 62 64 66 68 70 72 74 76 78 80 82 84 86 88 90 92 94
>E>T>H>C>H>Q>H>K>Y>C>D>P>N>L>G>L>R>V>Q>Q>K>G>T>S>E>T>D>T>I>C>T>C>E>E>G>W>



PfoI CspCI BmrI
GCACTGTACCTCCGAGGGGTGCGAGAGCTGTGTTCTCCACCGCTCTTGTCTCCCGGATTTGGGGTTAAACAAATAGCAACTGGGGTTTCAGATACAATATGCGAAC
96 98 100 102 104 106 108 110 112 114 116 118 120 122 124 126 128 130
>>H>C>T>S>E>A>C>E>S>C>V>L>H>R>S>C>S>P>G>F>G>V>K>Q>I>A>T>G>V>S>D>T>I>C>E>>

FIG. 10A (Cont'd)

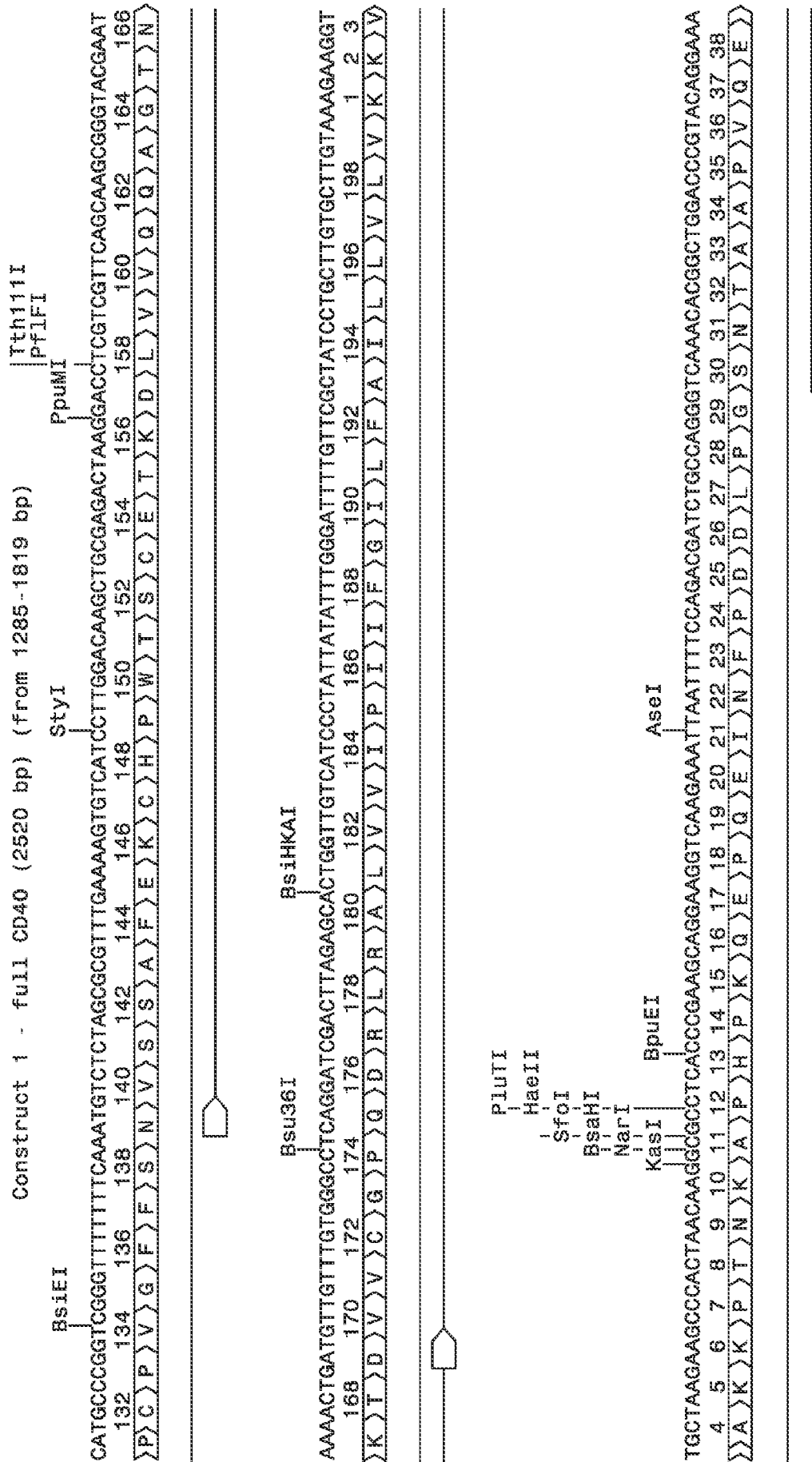


FIG. 10A (Cont'd)

EcoP15I
 7 8 9 A G S G A T N F S L L K Q A G D Y E E N P G P M V S K G E E D N M A
 GATTATGCAGGCTCTGGTGCTACTAATTTTCTCTGCTGAACAGGCAGGGGACGTCGAAGAGAACCCCTGGCCCCATGGTTTCTAAGGGGGAAGACACAACATGGC
 AatII
 ZraI
 BsaHI
 BsmFI
 StyI
 NcoI
 BtgI

FIG. 10A (Cont'd)

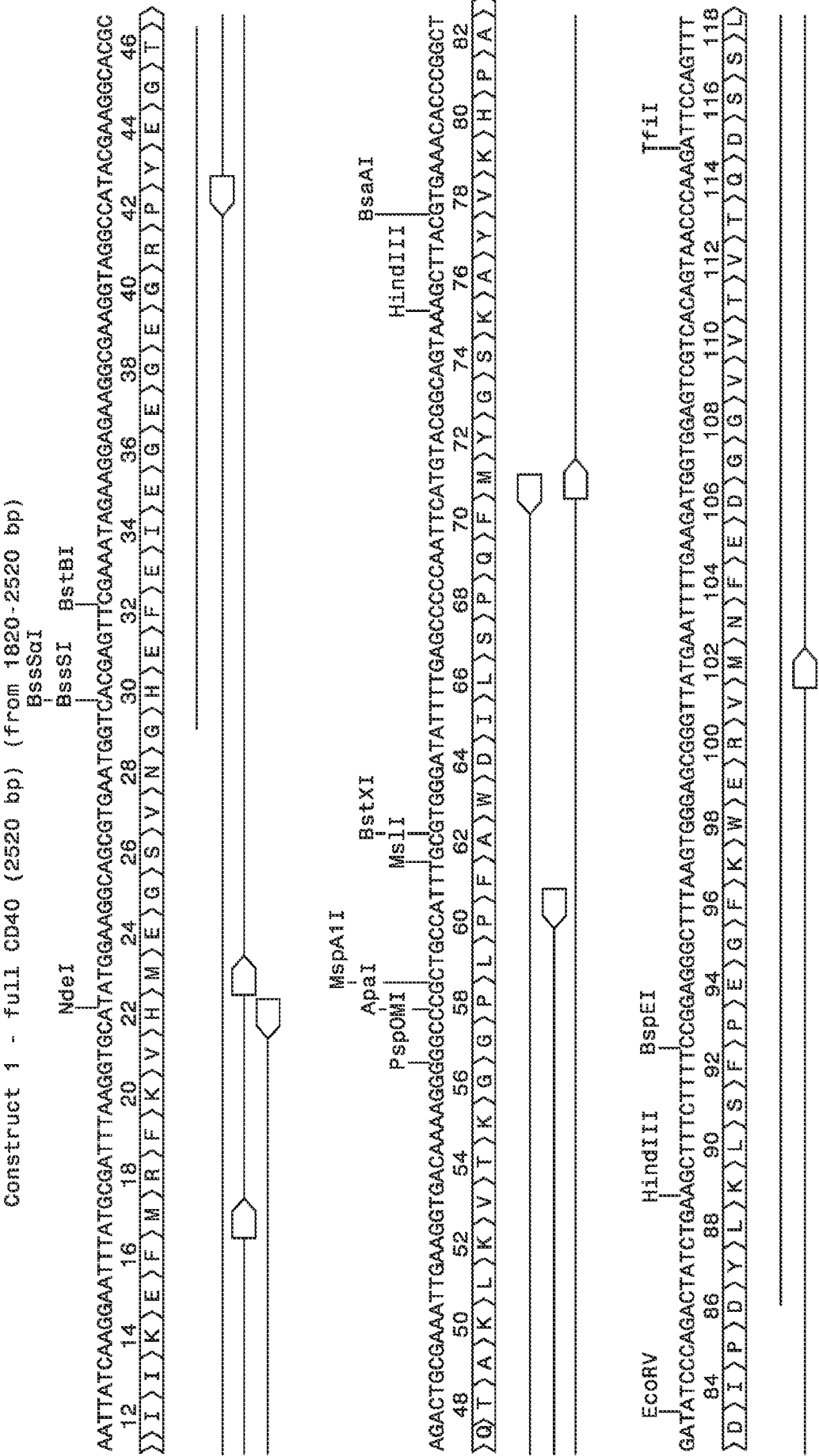


FIG. 10A (Cont'd)

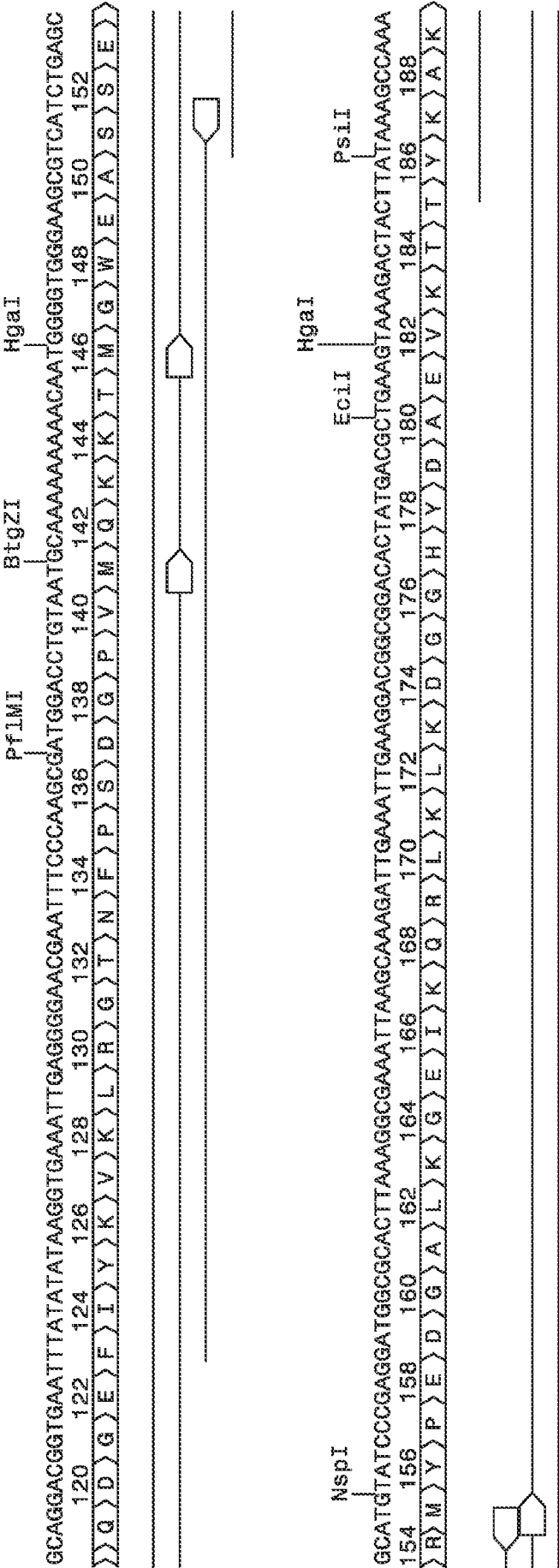


FIG. 10A (Cont'd)

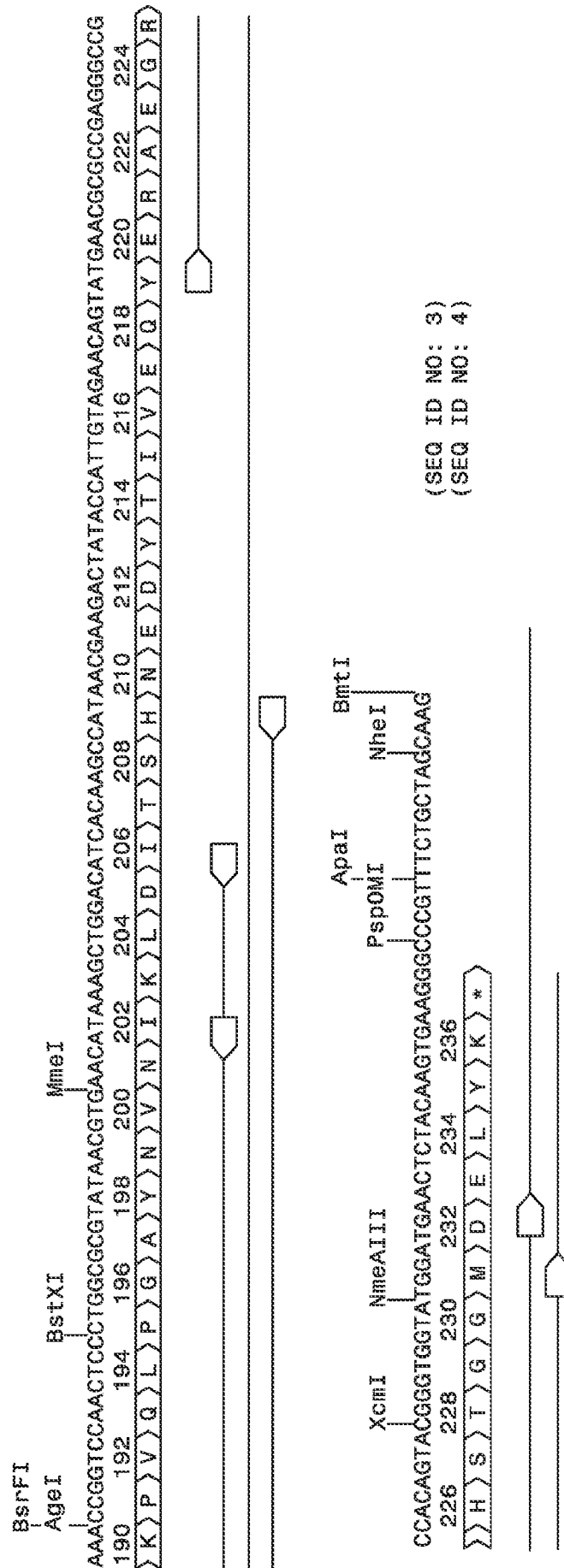


FIG. 10A (Cont'd)

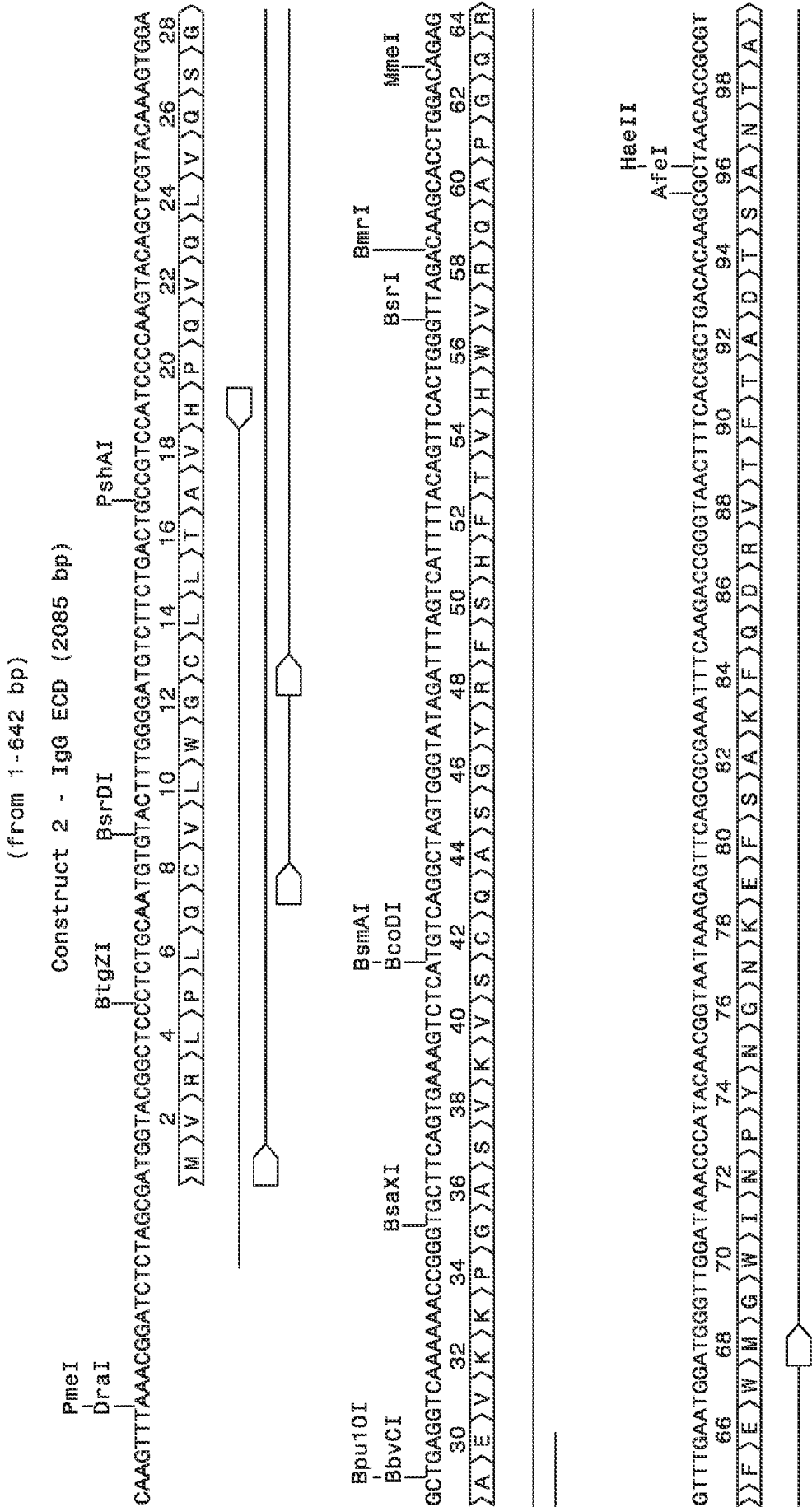


FIG. 10B

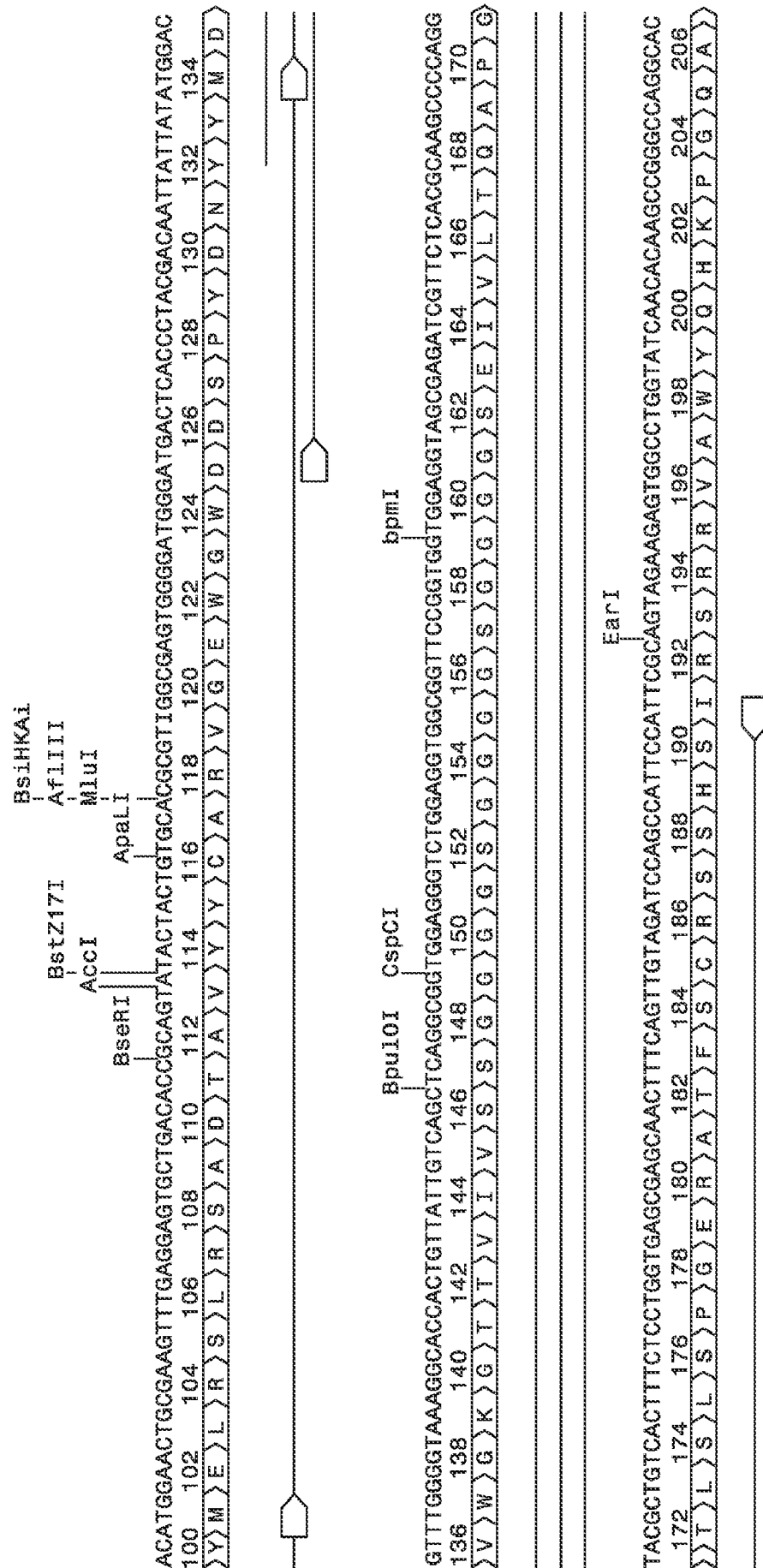


FIG. 10B (Cont'd)

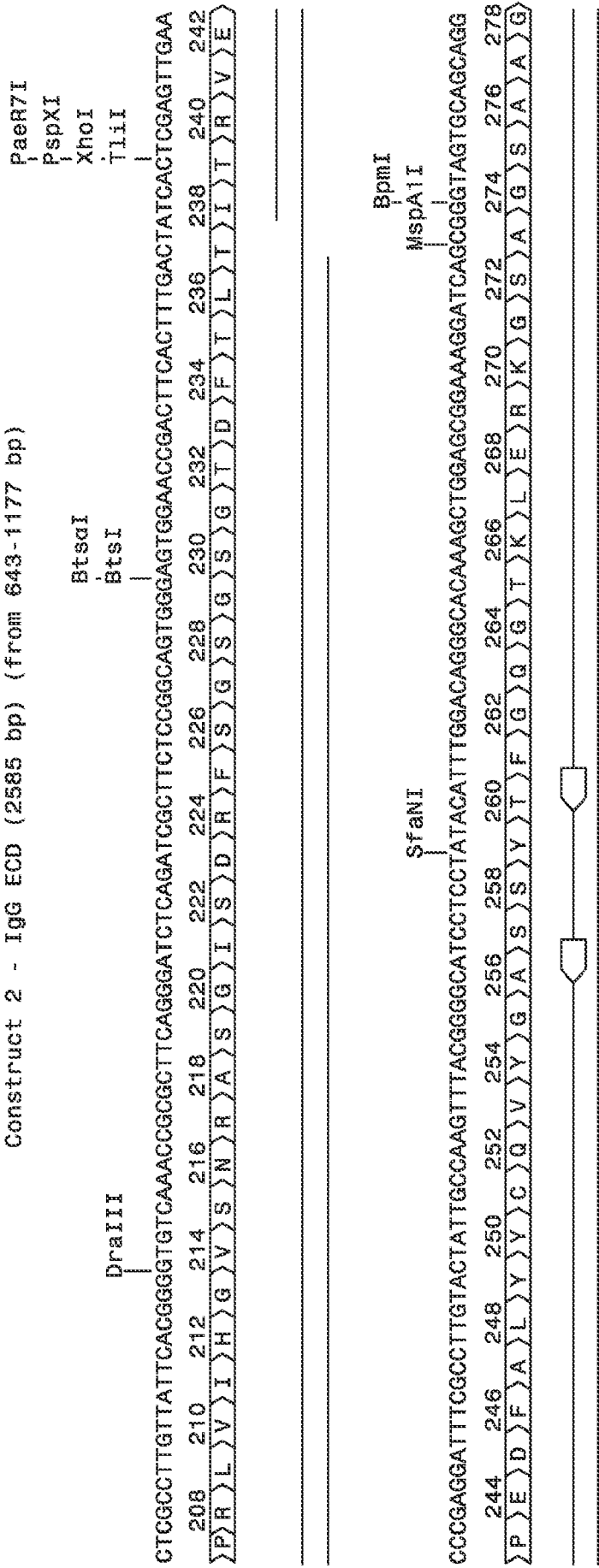


FIG. 10B (Cont'd)

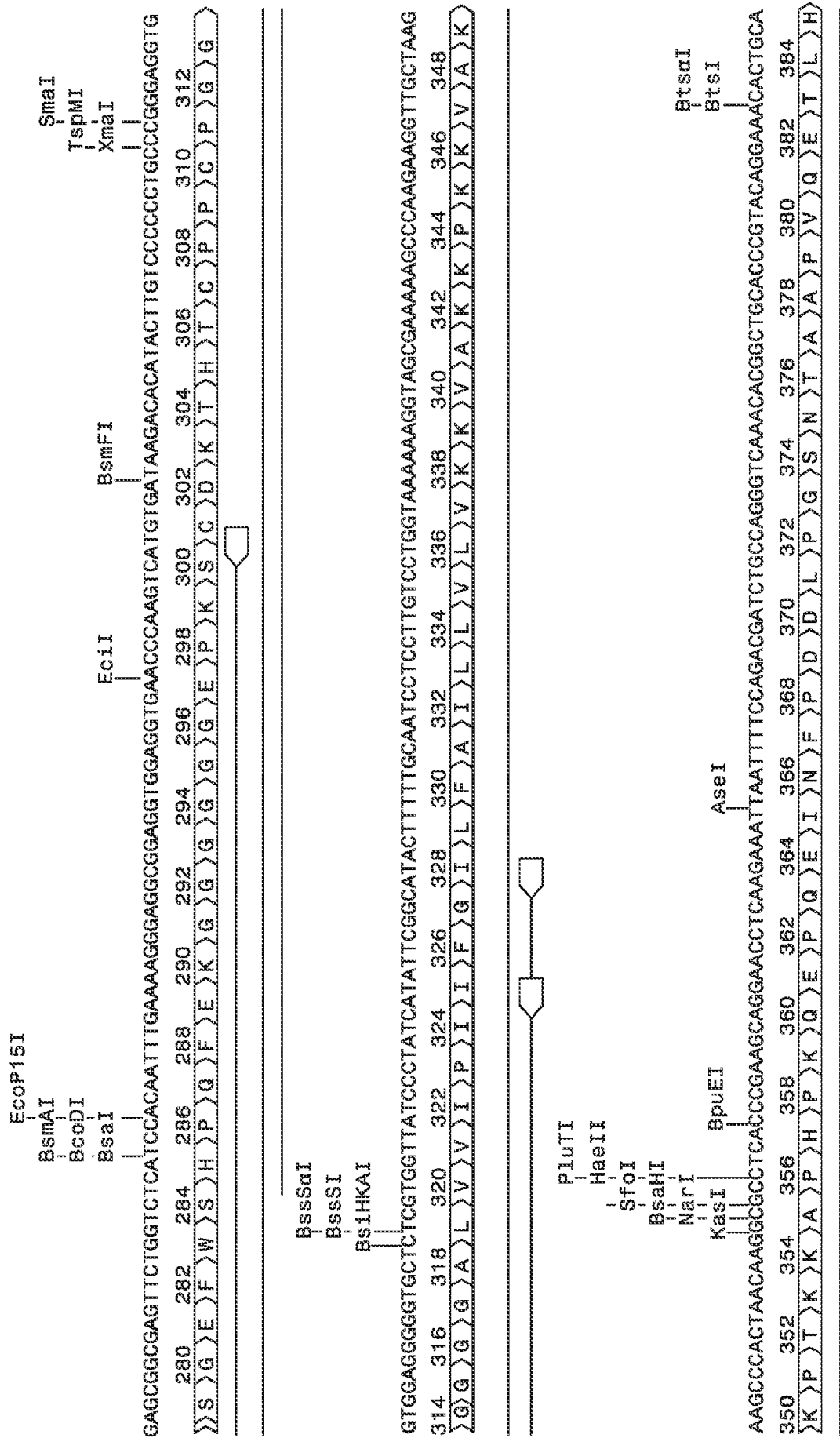
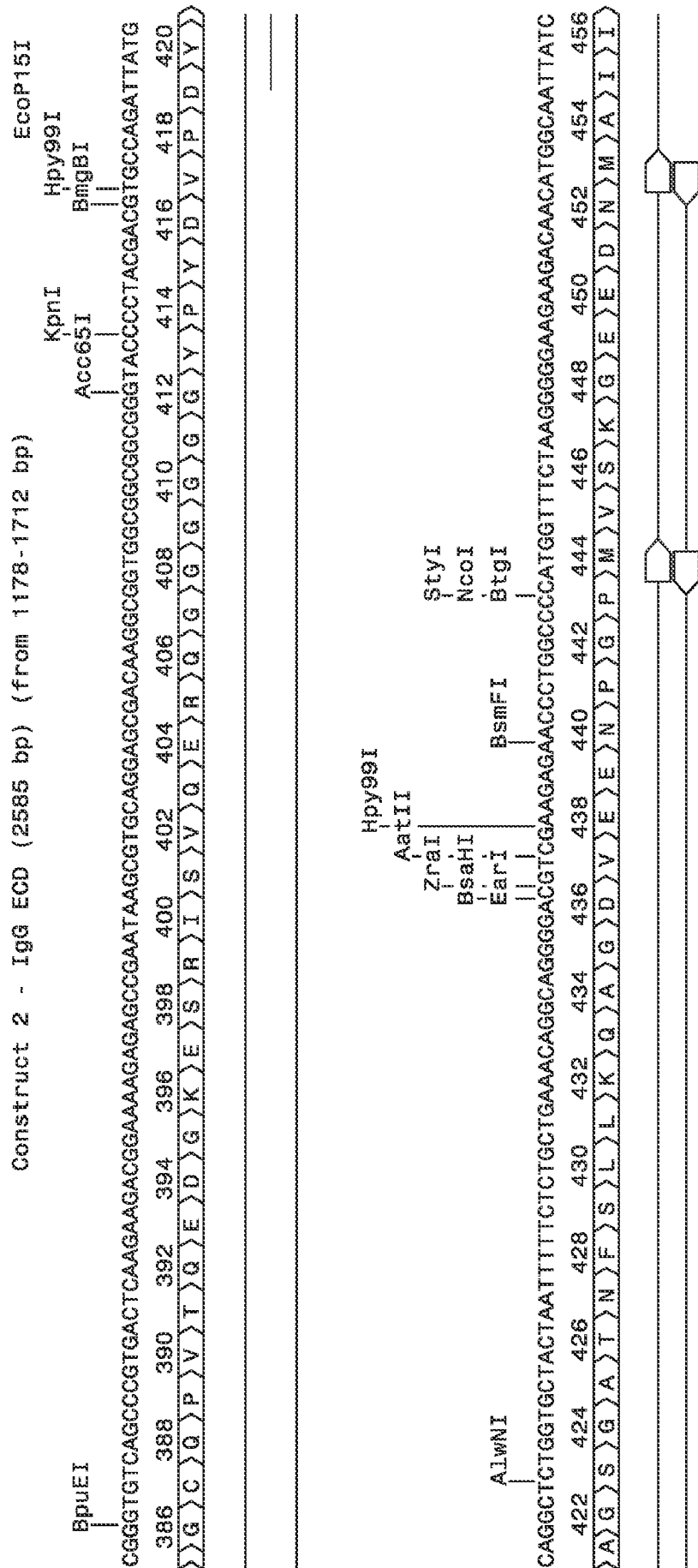


FIG. 10B (Cont'd)



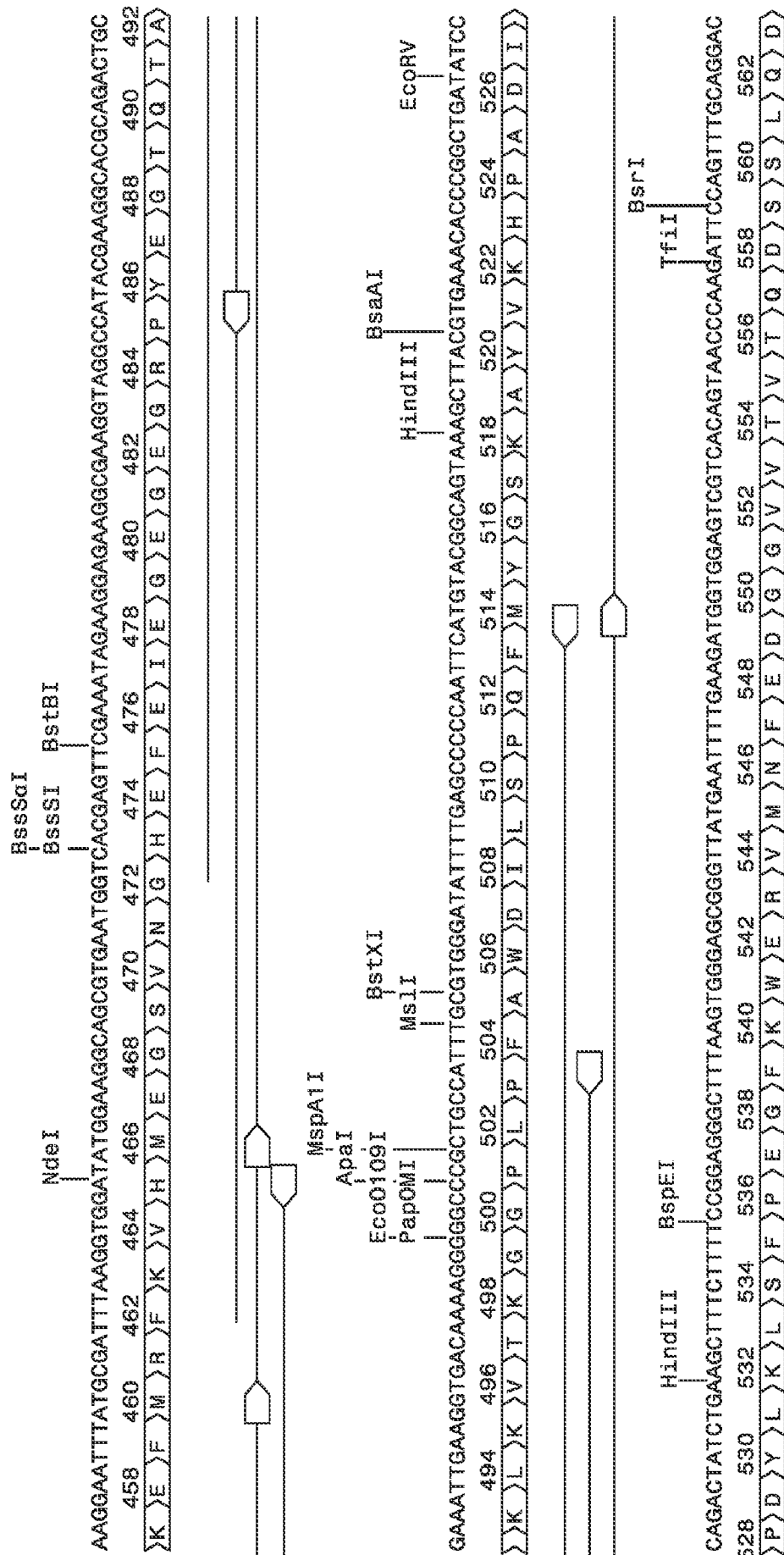


FIG. 10B (Cont'd)

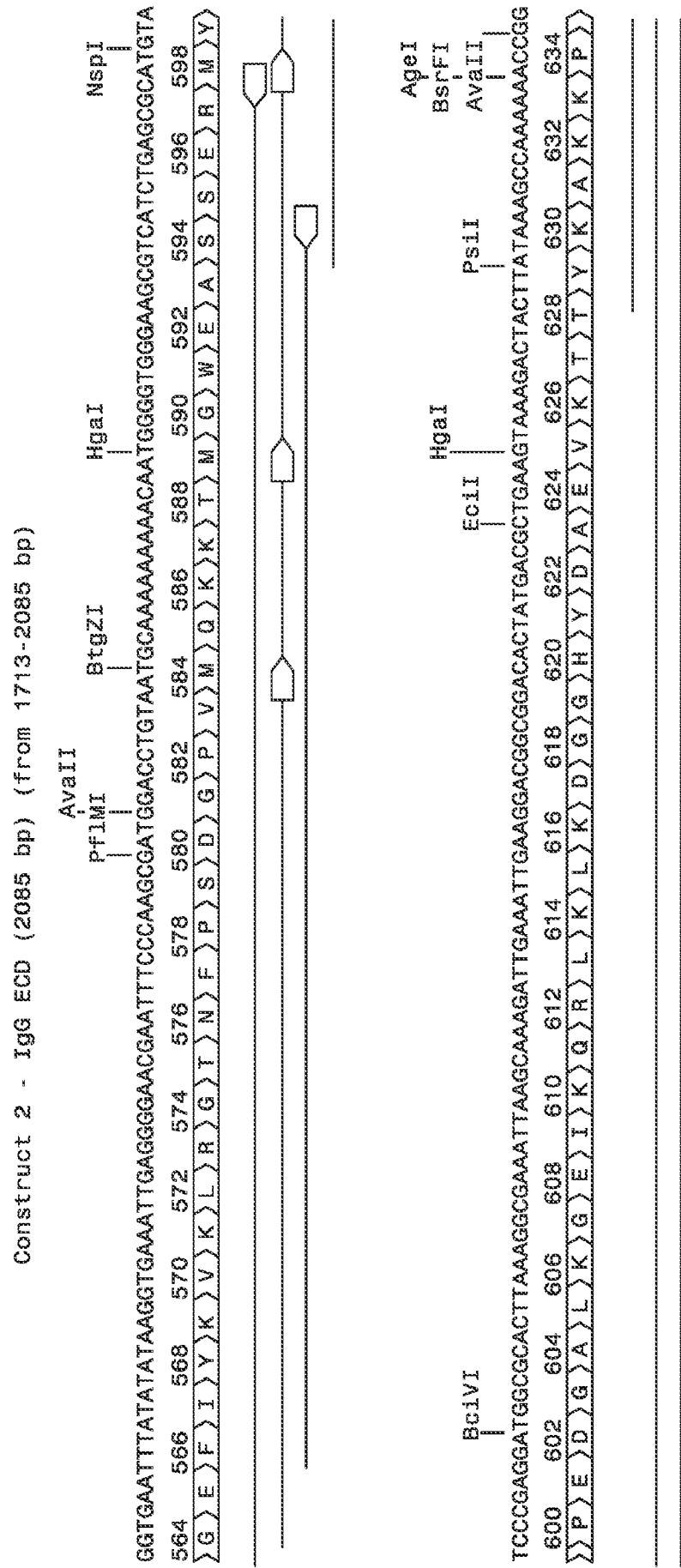


FIG. 10B (Cont'd)

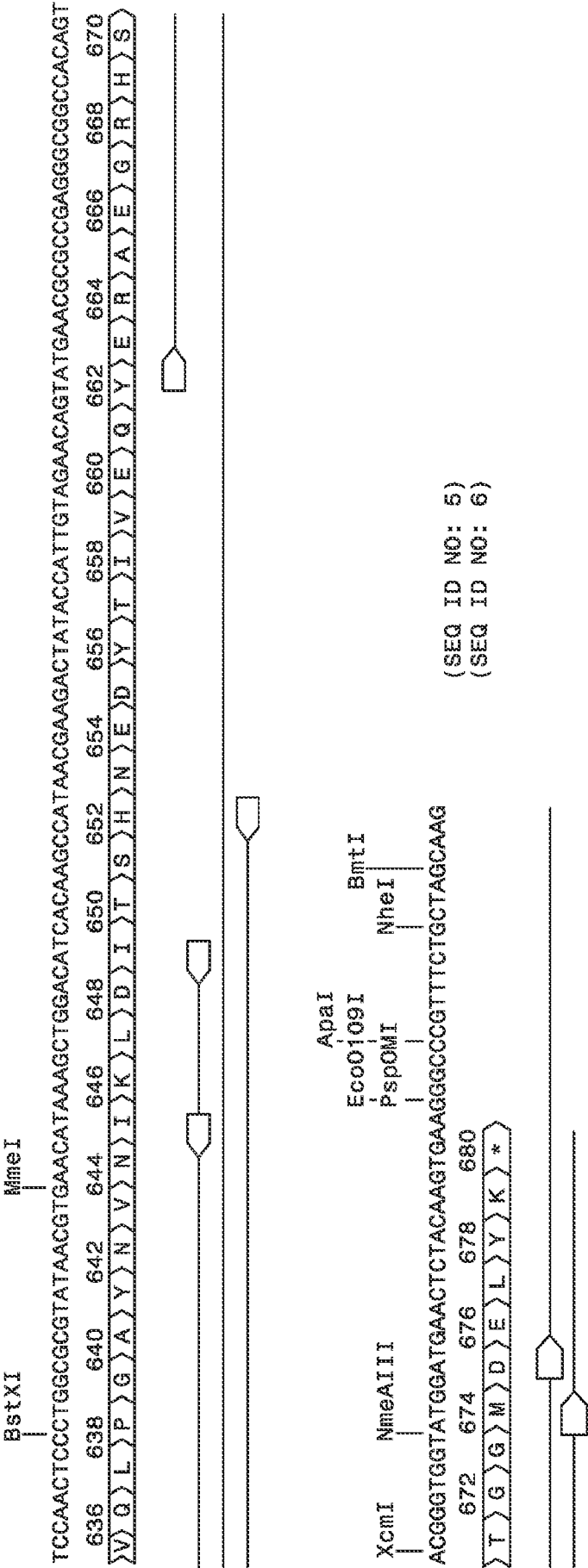


FIG. 10B (Cont'd)

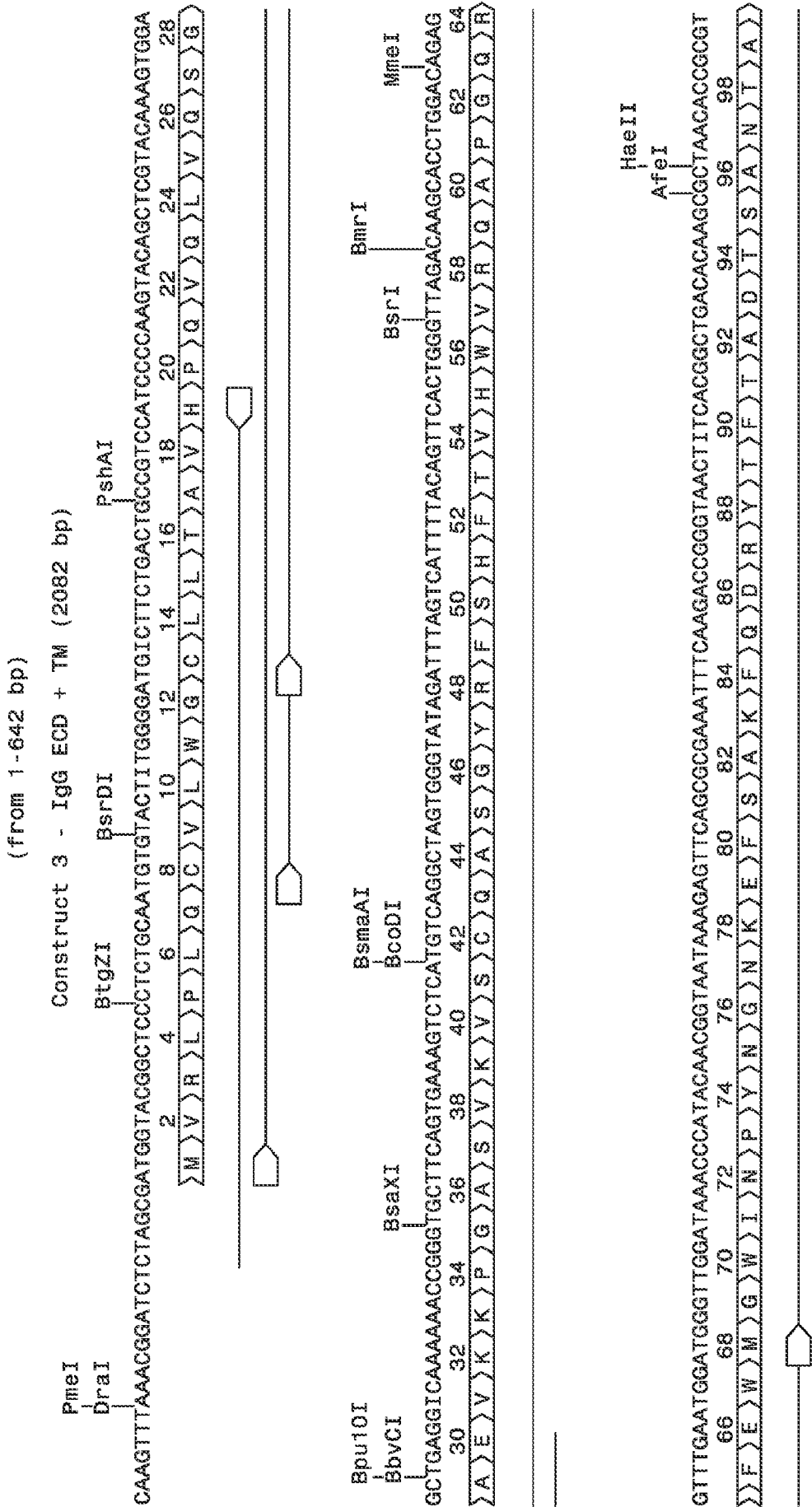


FIG. 10C

BstZ17I
ACCI
BseRI
BstZ17I
AflIII
MluI
BsiHKaI

ApalI

ACATGGAAGTTCGAGGAGTCTGACACCGCAGTATCTACTGTGCACGCGTIGGCGAGTGGGATGGATGACTCACCCCTACGACAATTATTATGAC
100 102 104 106 108 110 112 114 116 118 120 122 124 126 128 130 132 134
>Y>M>E>L>R>S>A>D>T>A>V>Y>Y>C>A>R>V>G>E>W>G>W>D>D>S>P>Y>D>N>Y>Y>M>D>

Bpu10I
GTTTGGGGTAAAGGCACCACTGTTATTGTCAGCTCAGGCGGTGGAGGGTCTGGAGGTGGCGGTTCCGGTGGTGGAGGTAGCCGAGATCGTTCTCAGCAAGCCCCAGG
136 138 140 142 144 146 148 150 152 154 156 158 160 162 164 166 168 170
>V>W>G>K>G>T>T>V>I>V>S>S>G>G>G>G>S>G>G>G>S>G>G>G>S>E>I>V>L>T>Q>A>P>G>

CspCI

BpmI

EaRI
TAGCTGICACTTICTCCIGGTGAGCGAGCAACTTTCAGTTGTAGATCCAGCCATTCCATTCCAGTAGAAGAGIGGCCCTGGTATCAACACAAGCCGGCCAGGCAC
172 174 176 178 180 182 184 186 188 190 192 194 196 198 200 202 204 206
>T>L>S>L>S>P>G>E>R>A>T>F>S>C>R>S>S>H>S>I>R>S>R>R>V>A>W>Y>Q>H>K>P>G>Q>A>

EaRI

FIG. 10C (Cont'd)

SUBSTITUTE SHEET (RULE 26)

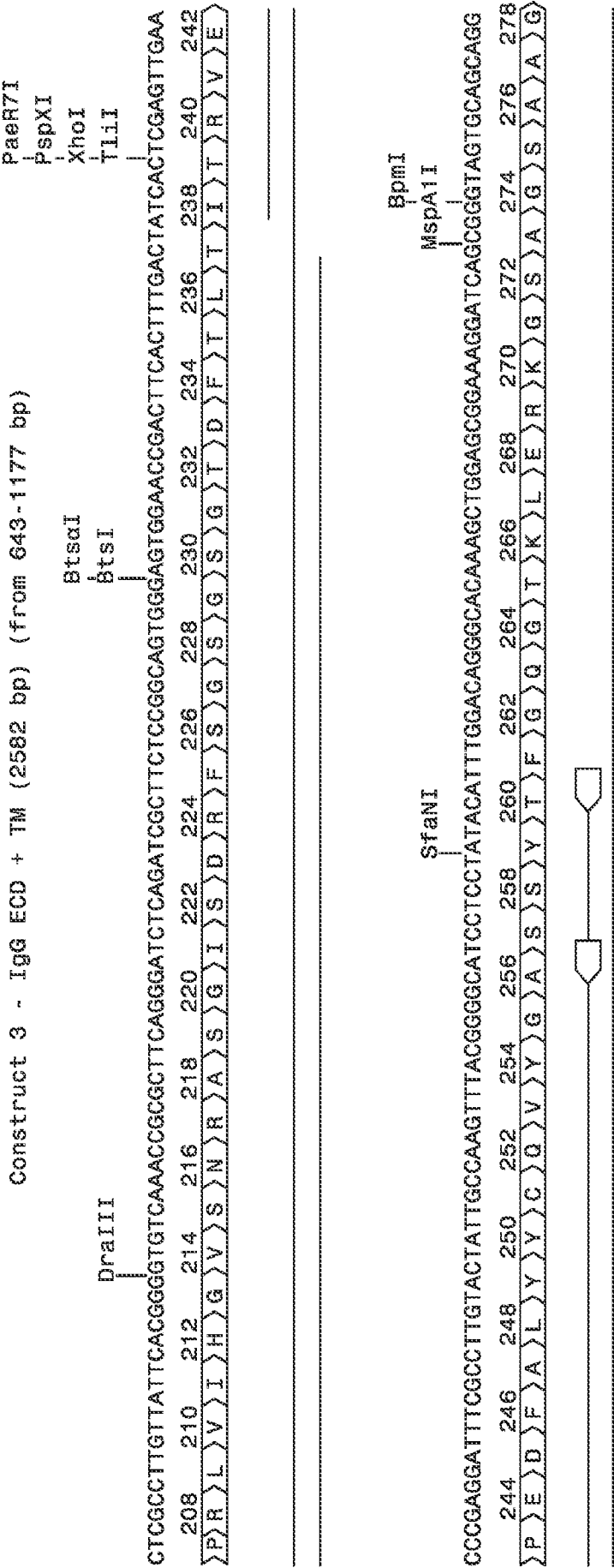
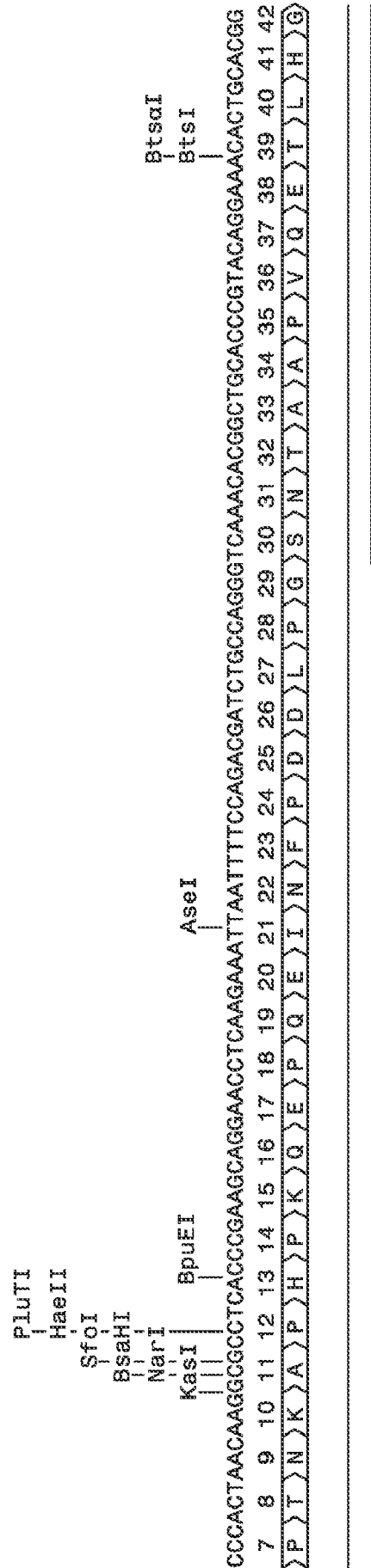


FIG. 10C (Cont'd)



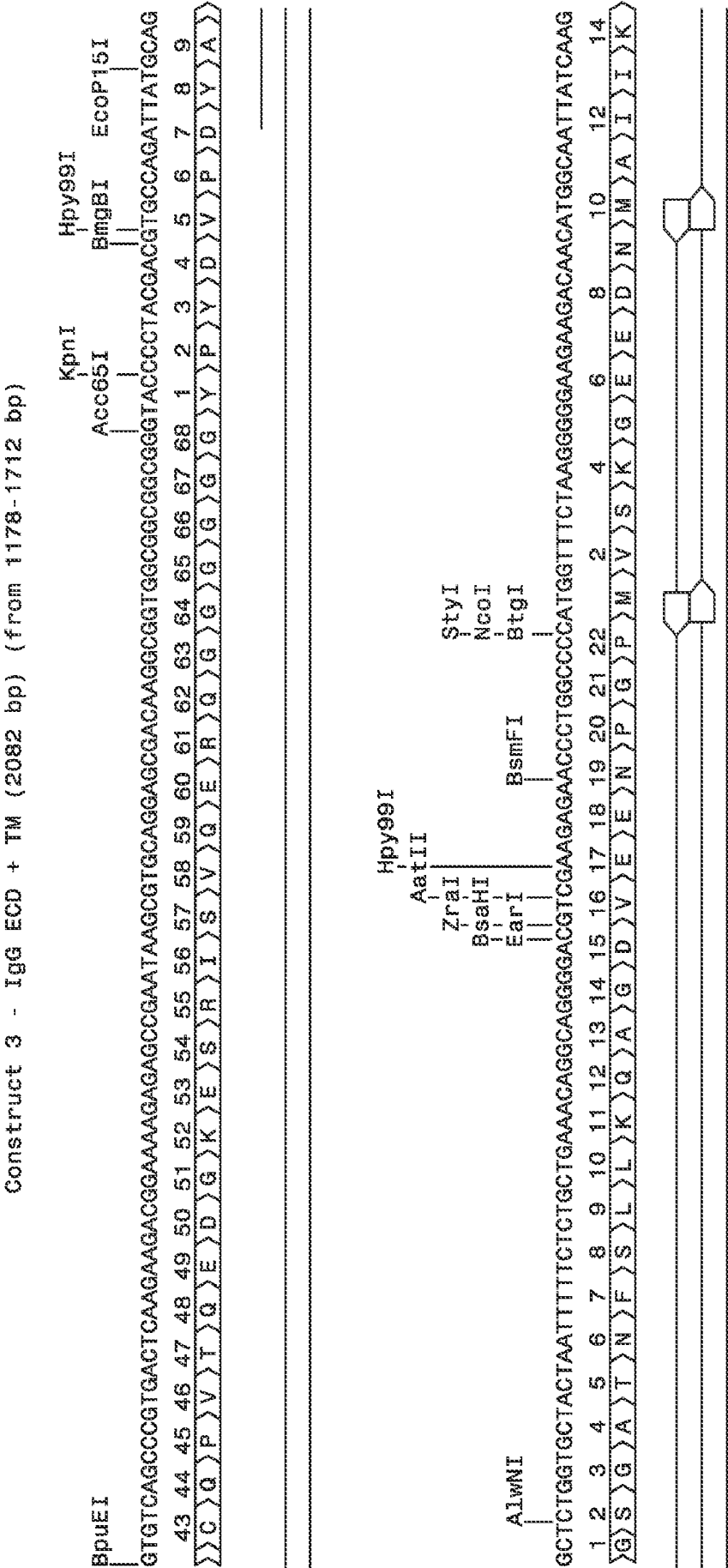


FIG. 10C (Cont'd)

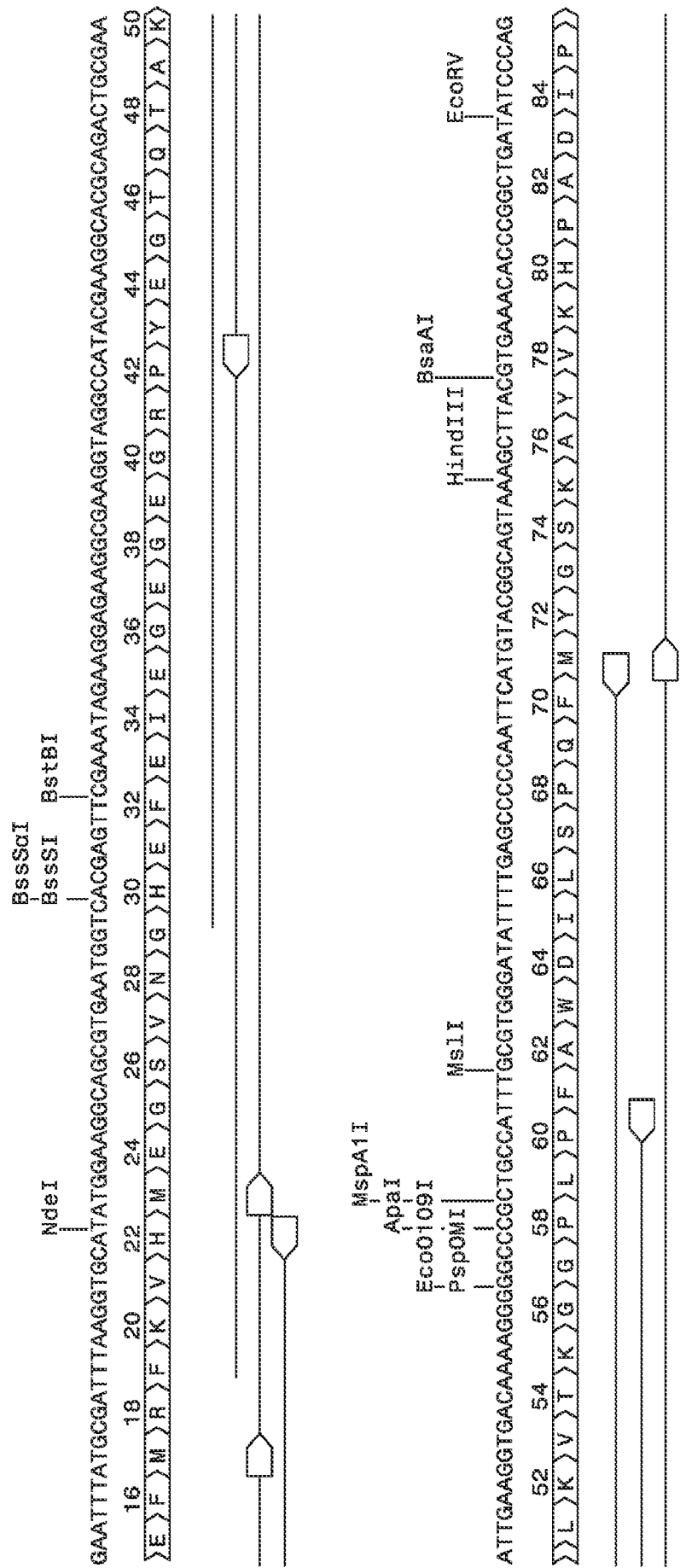


FIG. 10C (Cont'd)

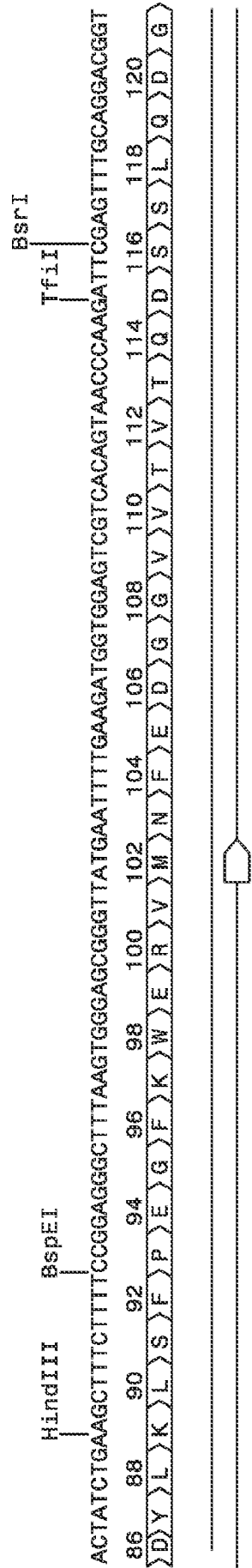


FIG. 10C (Cont'd)

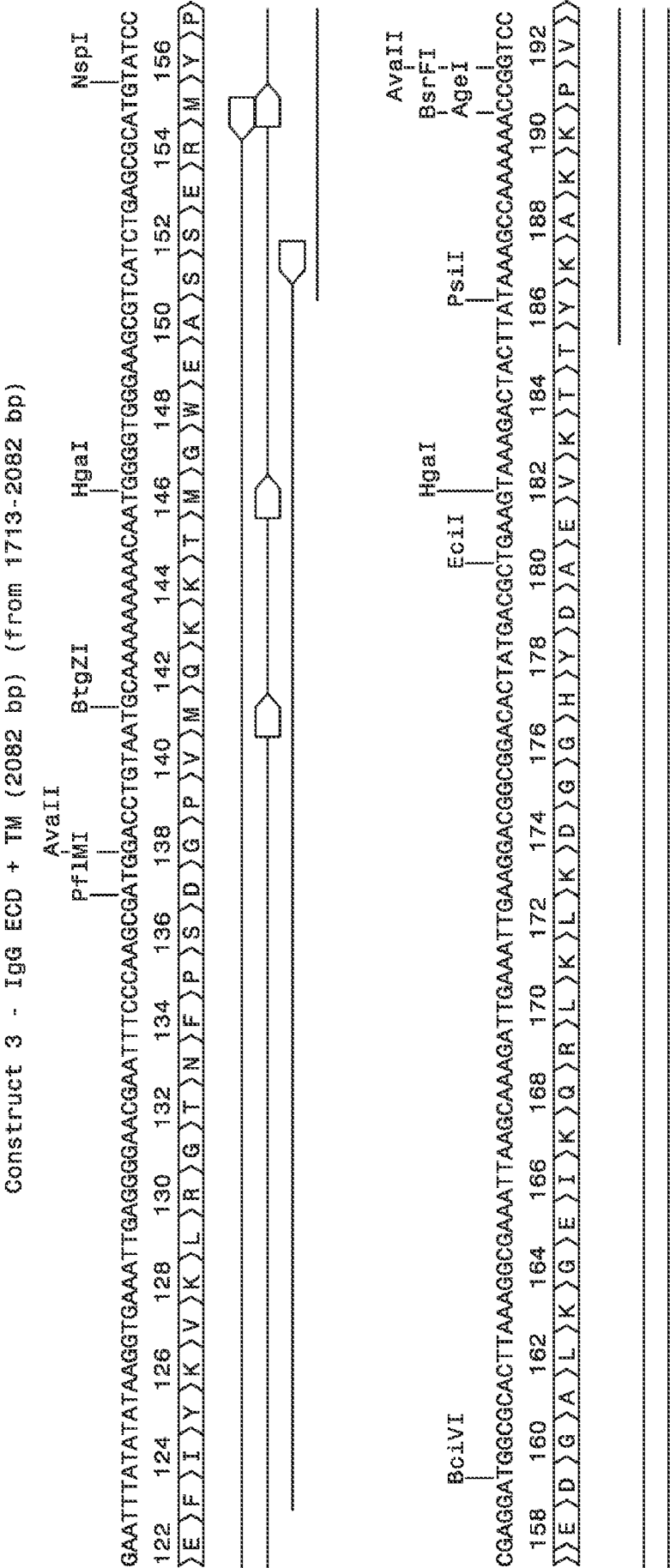


FIG. 10C (Cont'd)

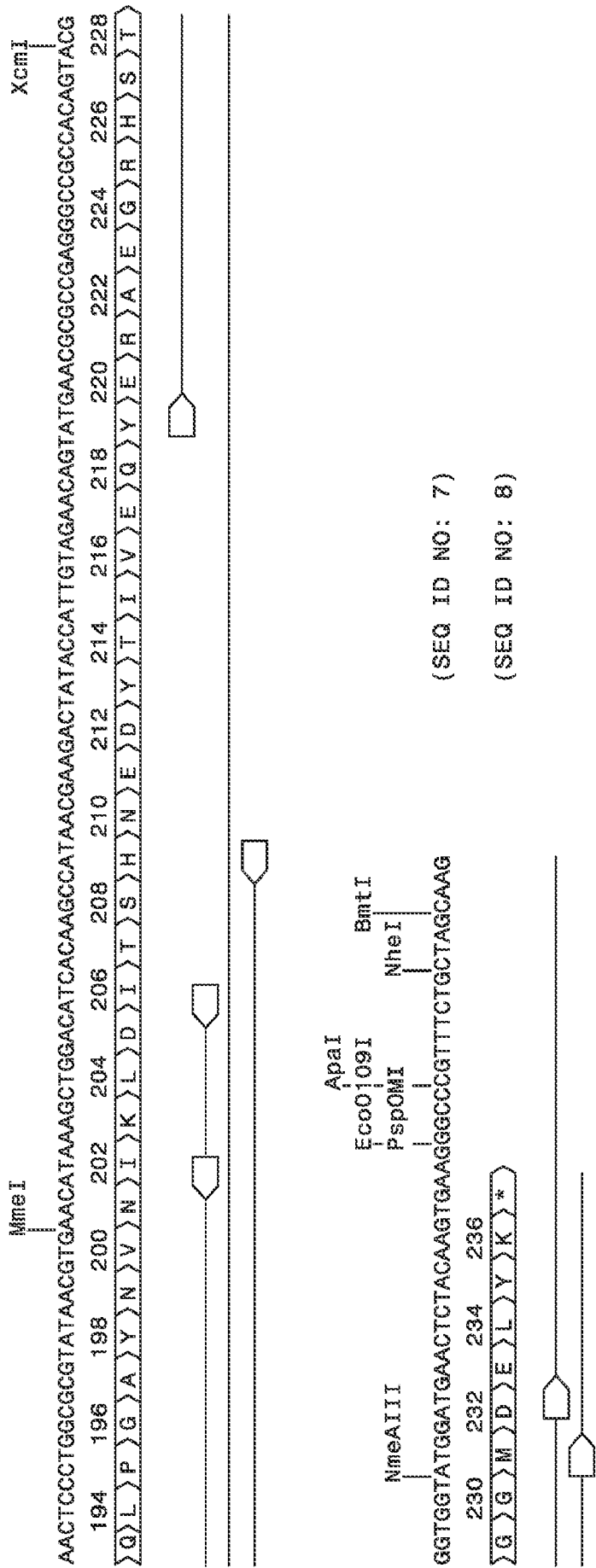


FIG. 10C (Cont'd)

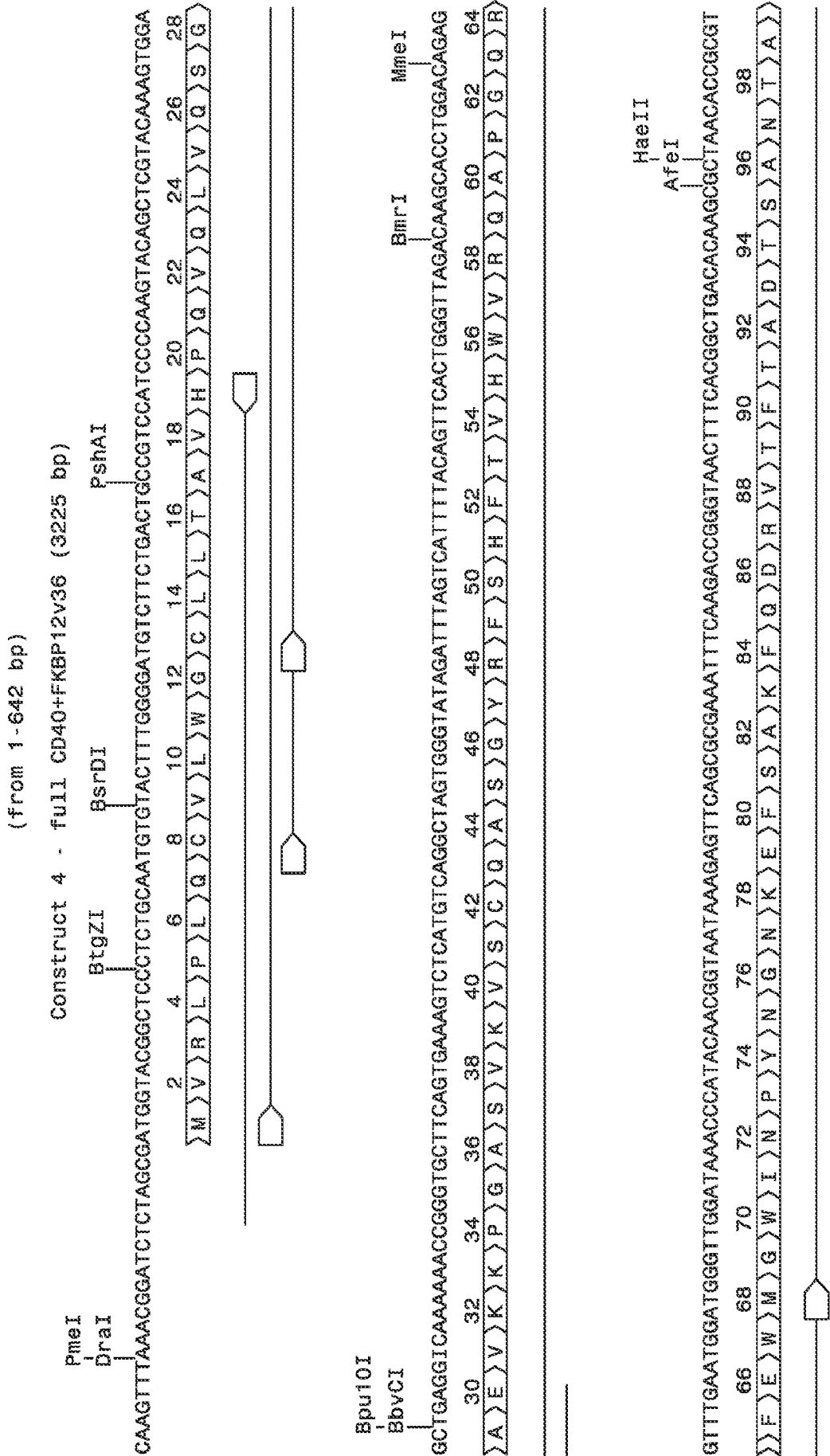
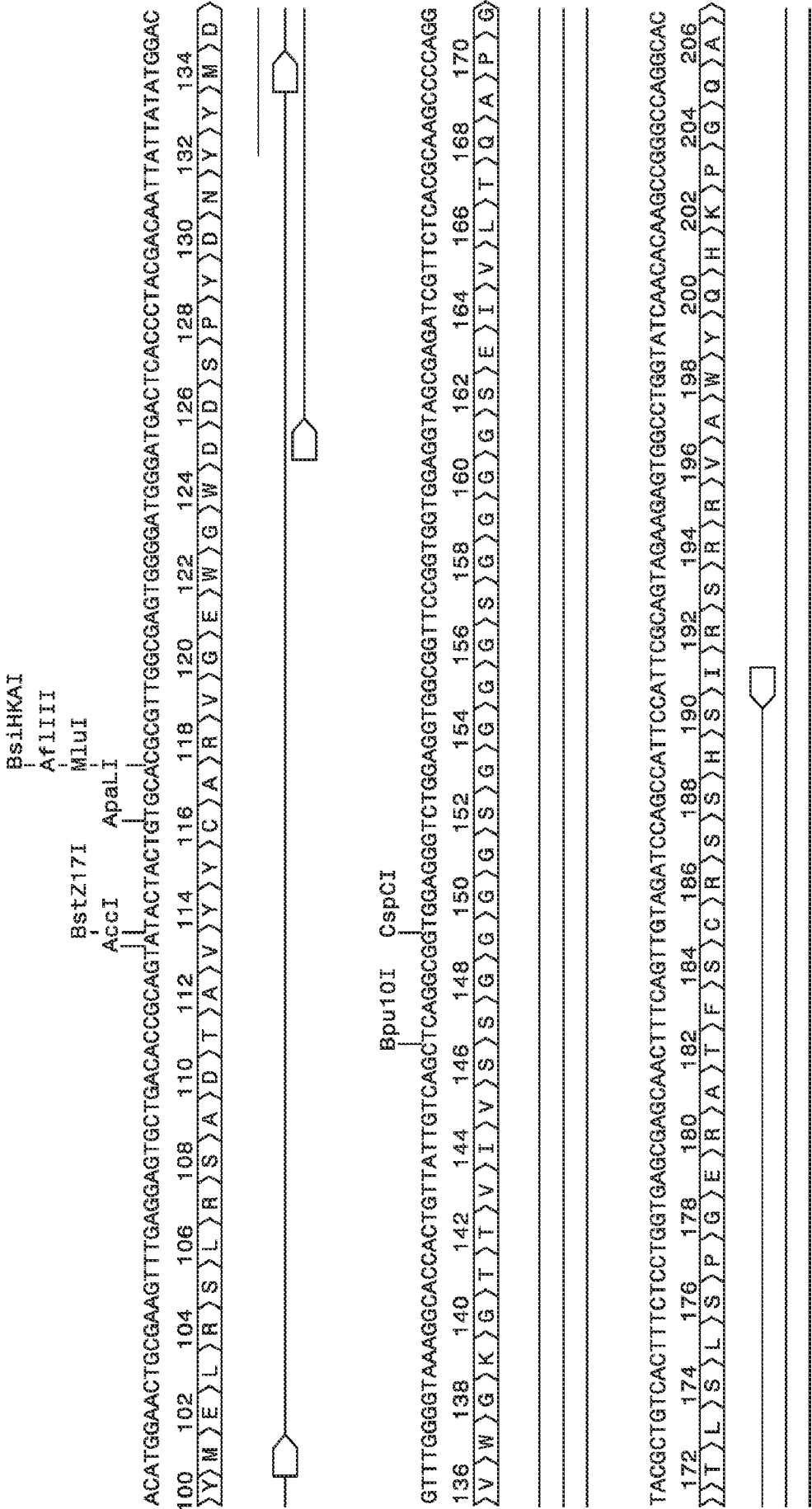


FIG. 10D



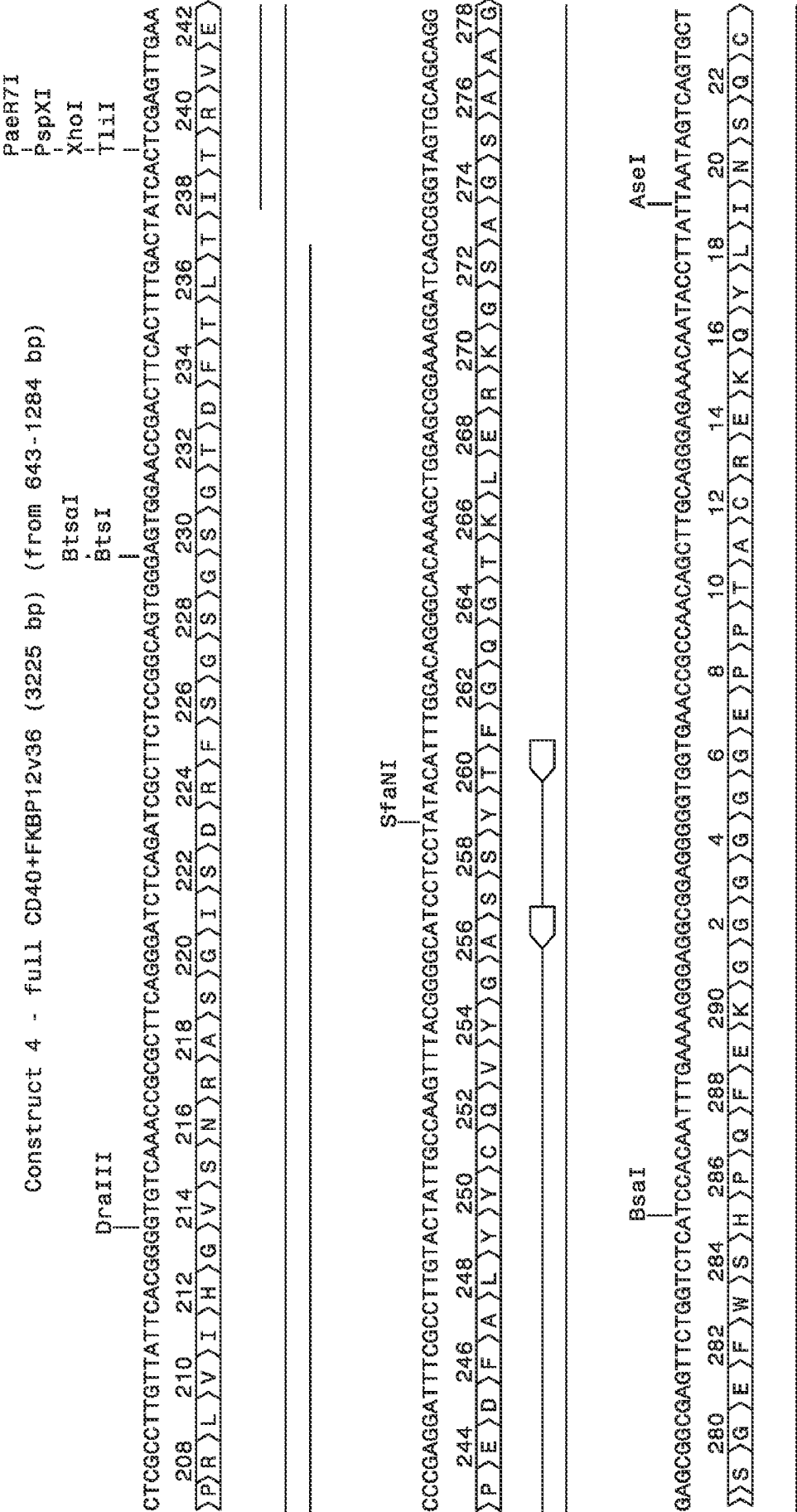


FIG. 10D (Cont'd)

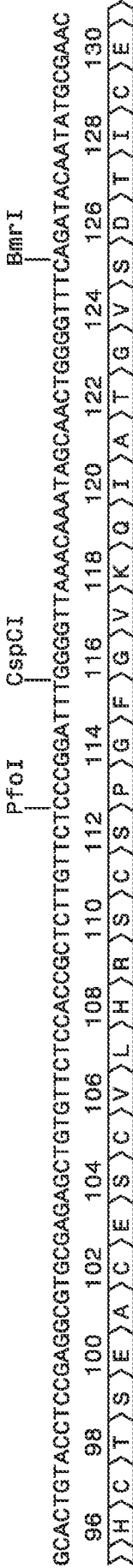
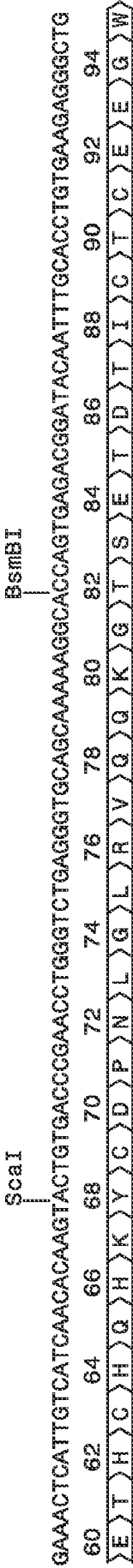
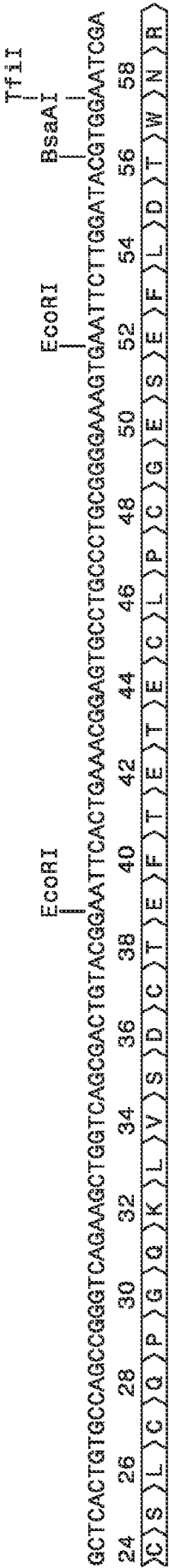


FIG. 10D (Cont'd)

CATGCCCGGTCGGGTTTTTTTCAAAATGTCCTCAGCGGTTTGAAAAGTGTTCATCCTTGGACAAGCTGCGAGACTAAGGACCTCGTCGTTCAGCAAGCGGGTACGGAAT

168	170	172	174	176	178	180	182	184	186	188	190	192	194	196	198	1	2	3																			
K	T	D	V	V	C	G	P	Q	D	R	L	R	A	L	V	V	I	P	I	I	F	G	I	L	F	A	I	L	L	V	L	V	L	V	K	K	V

SUBSTITUTE SHEET (RULE 26)

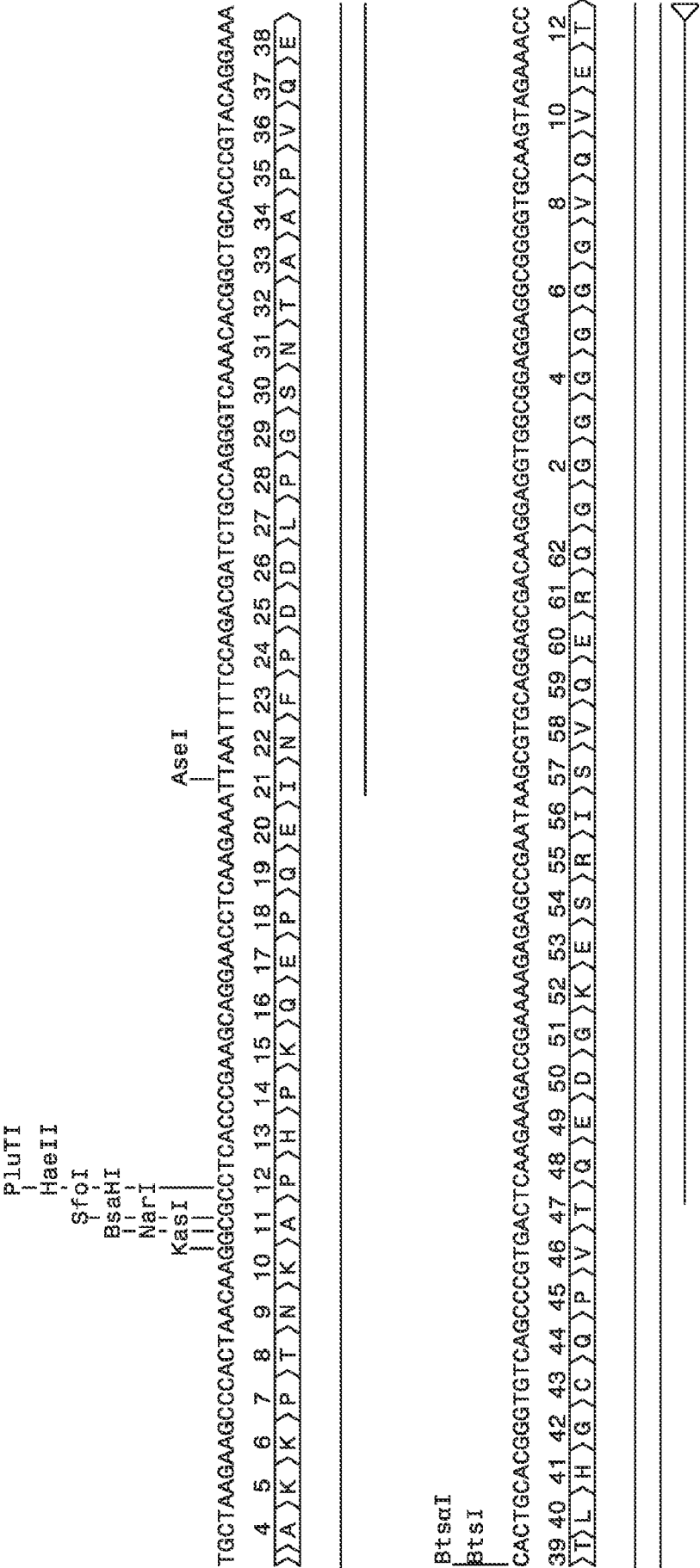


FIG. 10D (Cont'd)

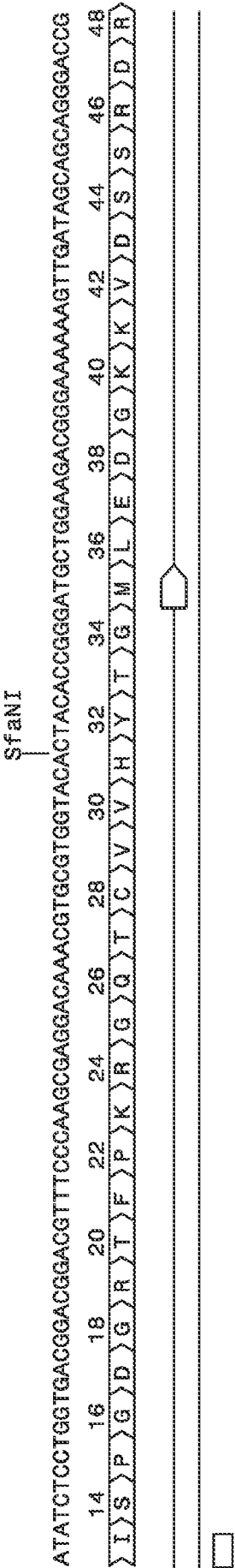


FIG. 10D (Cont'd)

Construct 4 - full CD40+FKBP12v36 (3225 bp) (from 1820-2461 bp)

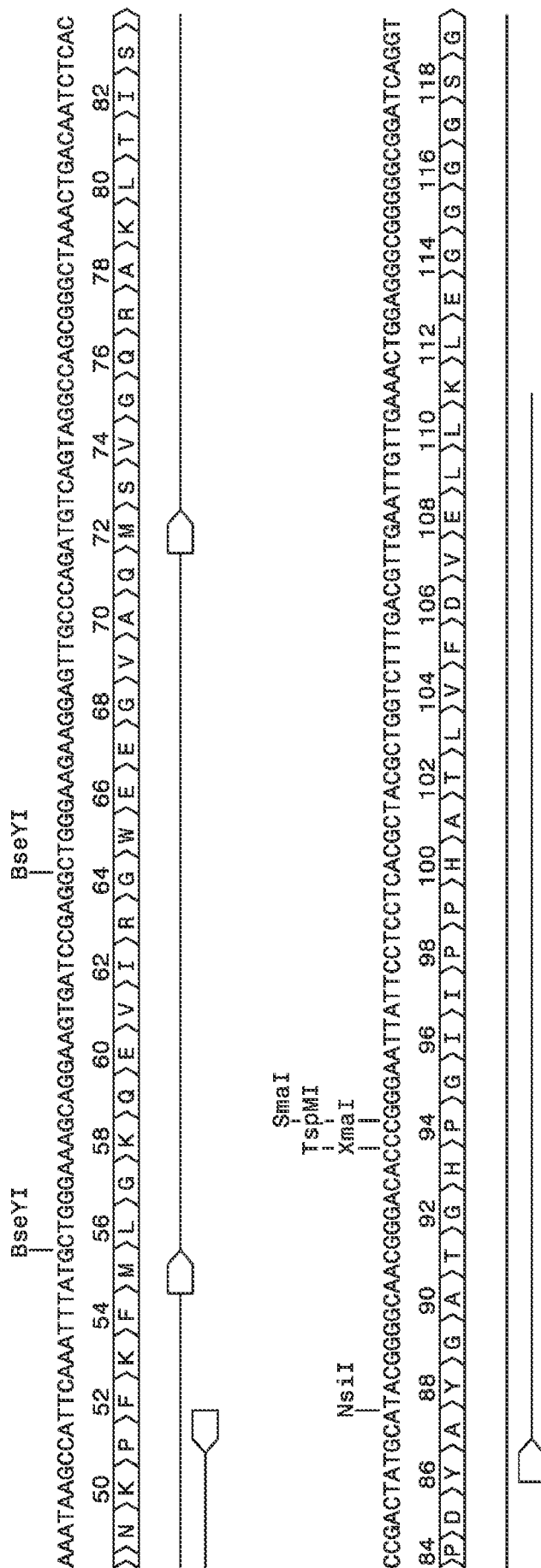


FIG. 10D (Cont'd)

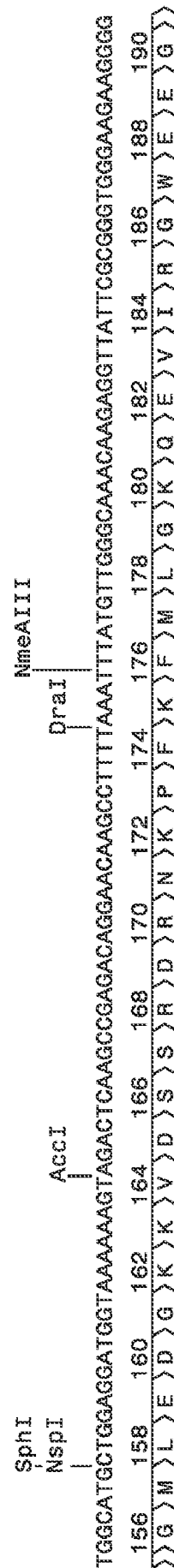
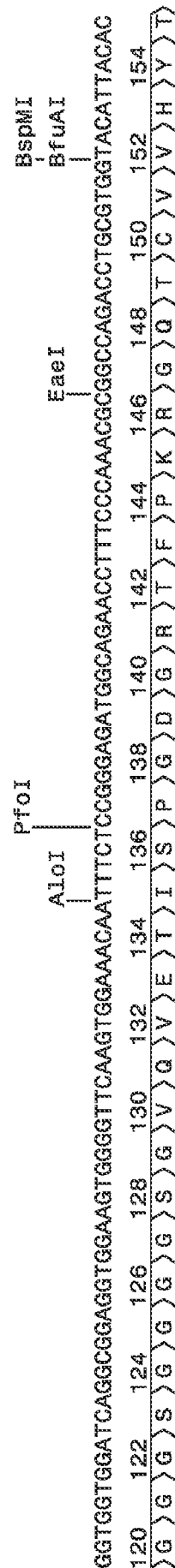


FIG. 10D (Cont'd)

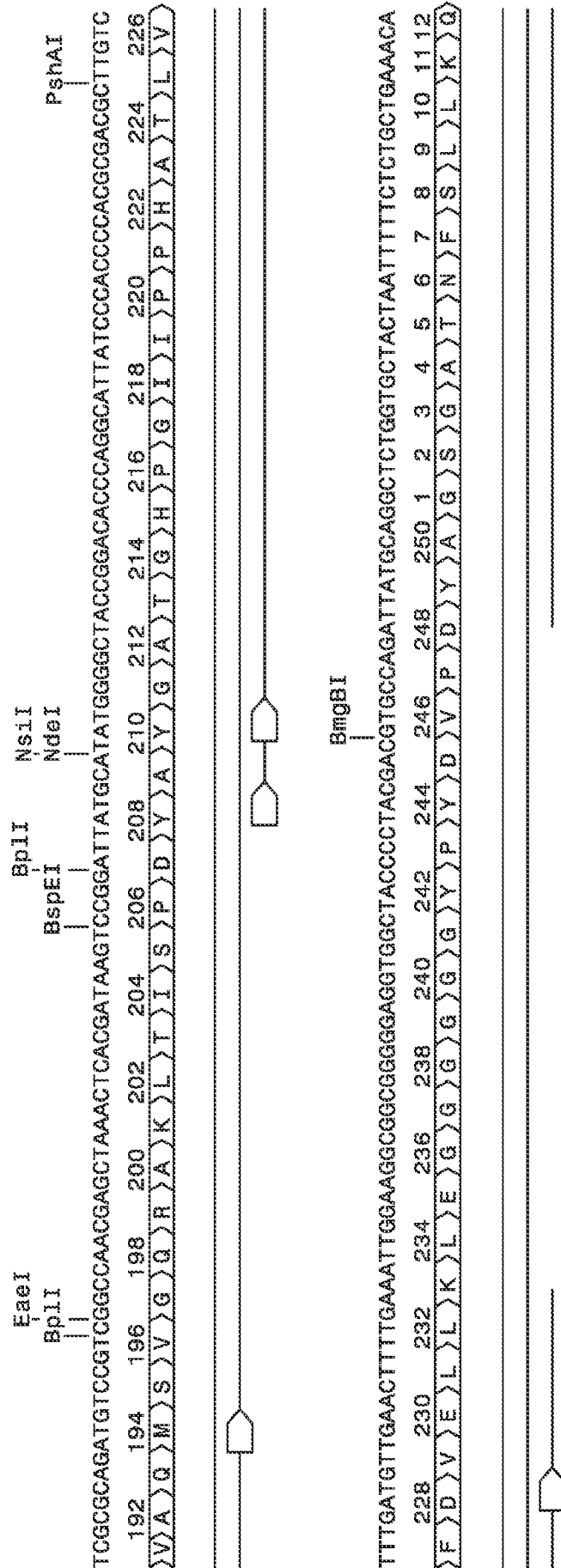
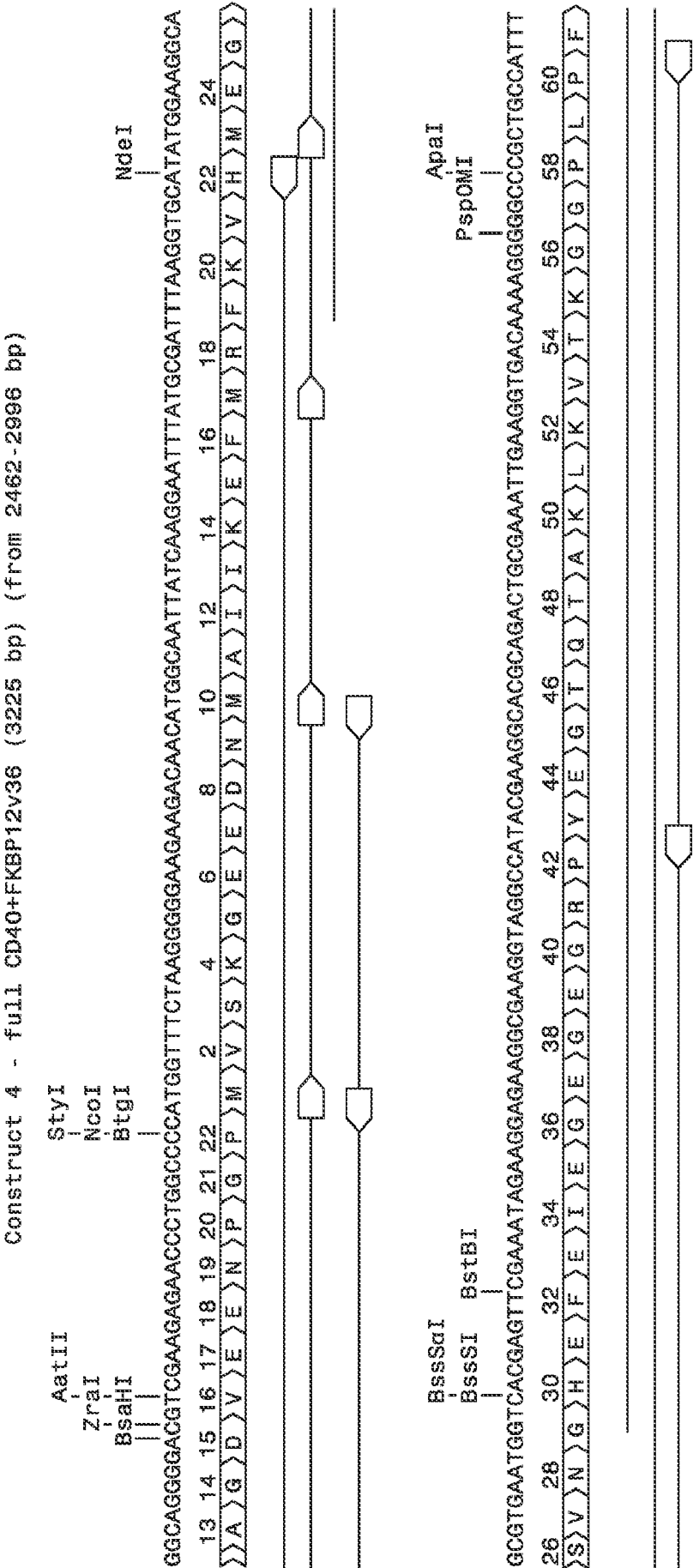


FIG. 10D (Cont'd)



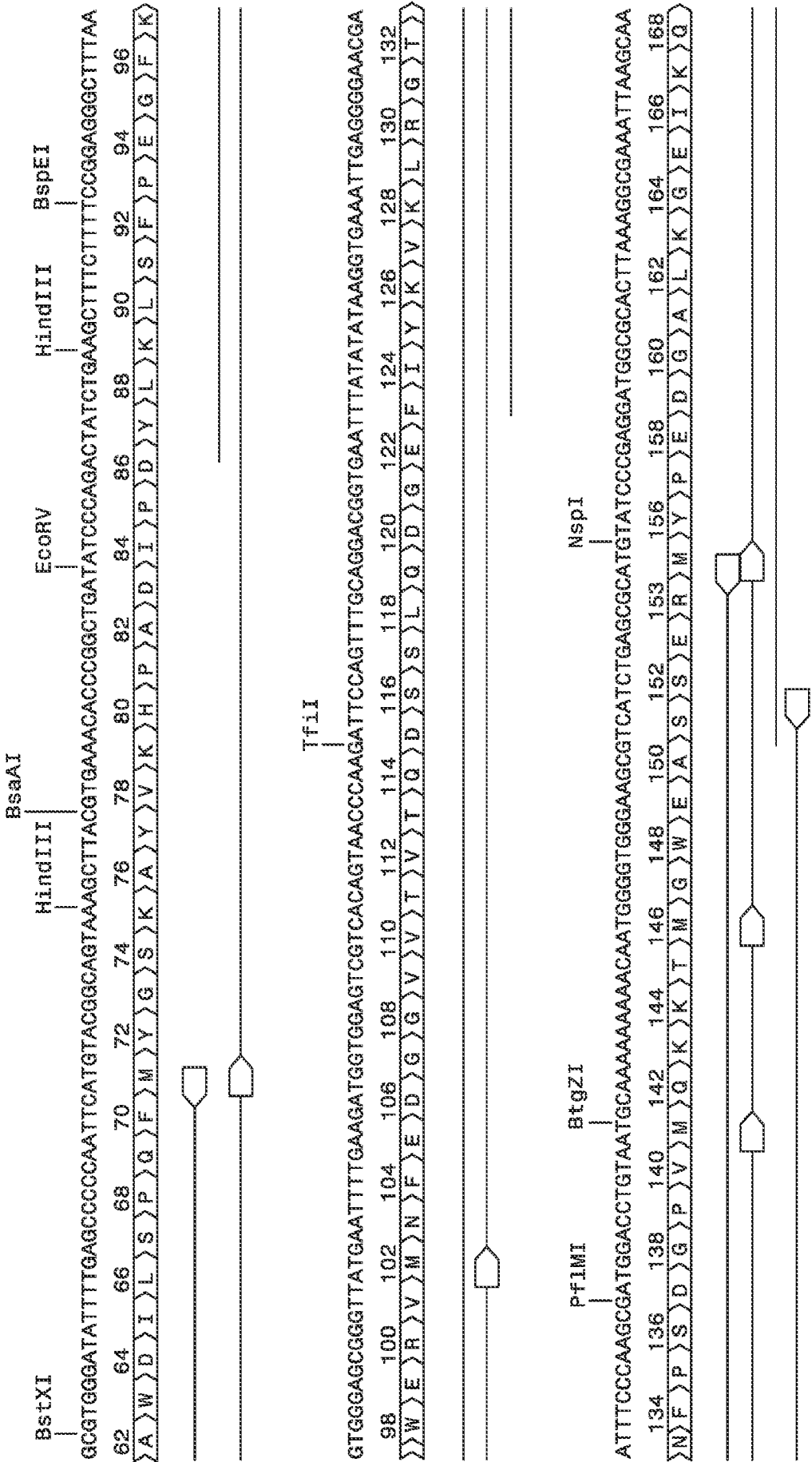


FIG. 10D (Cont'd)

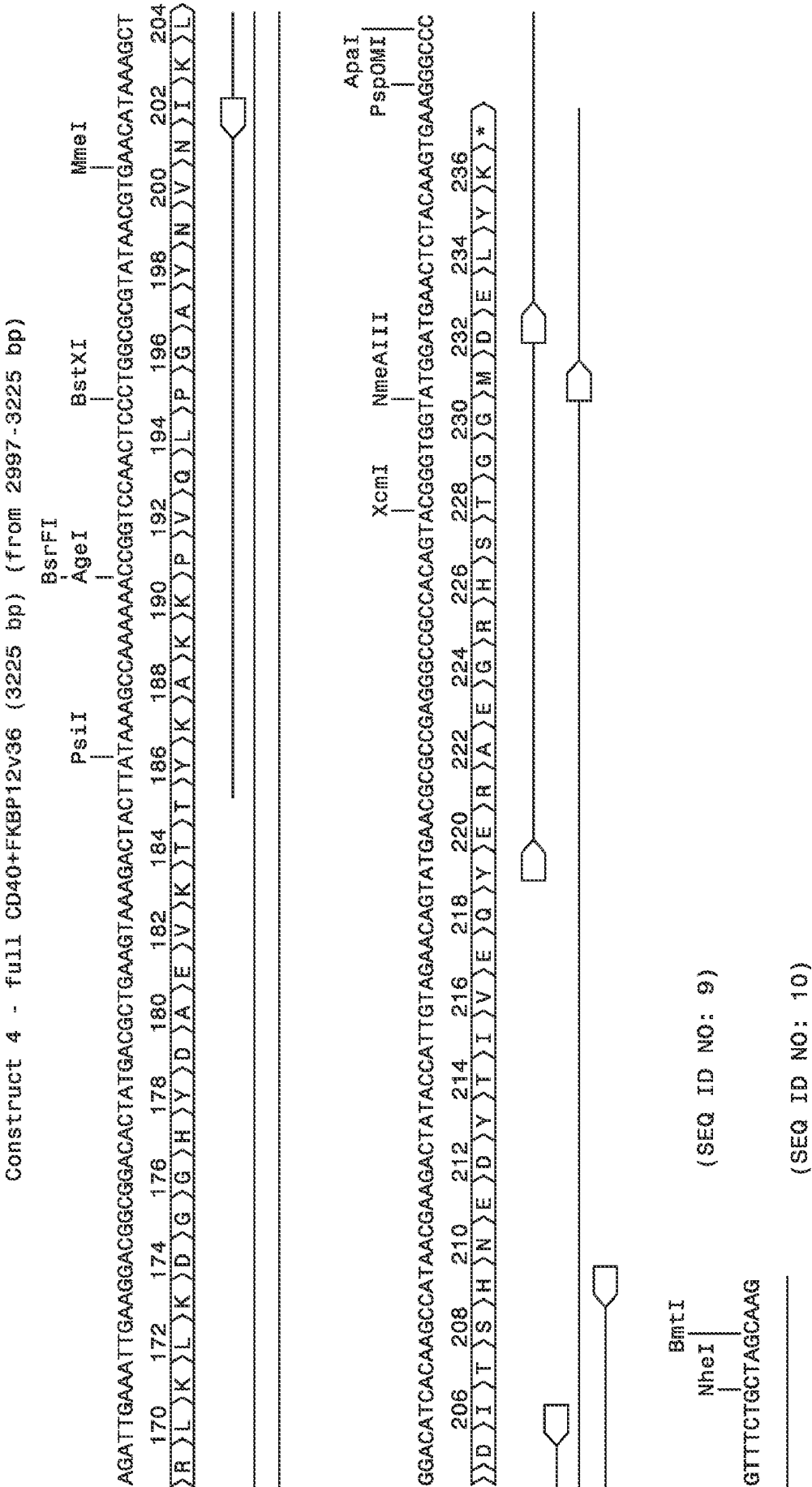


FIG. 10D (Cont'd)

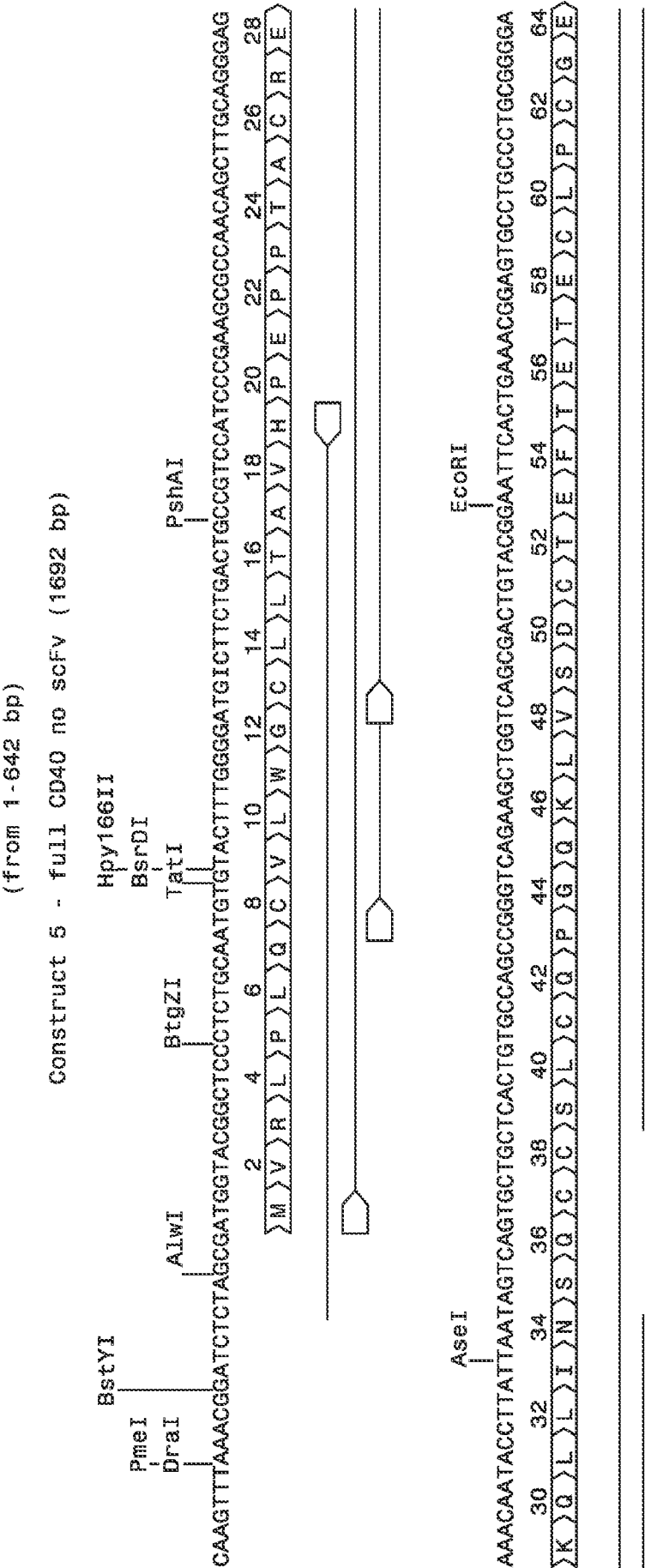


FIG. 10E

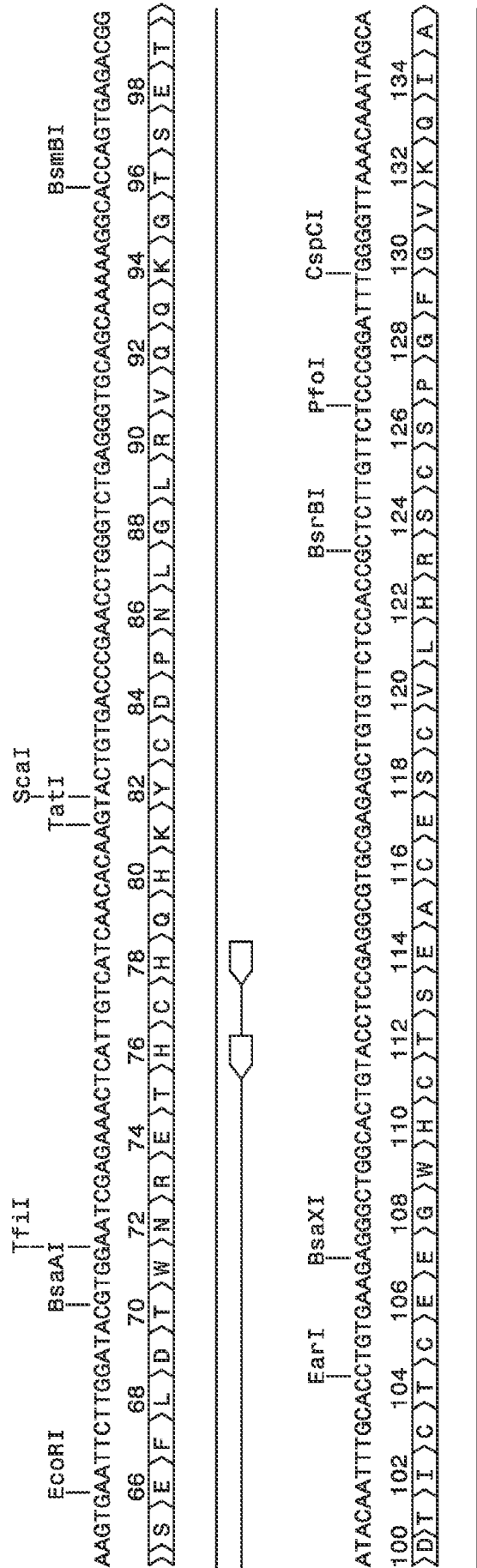


FIG. 10E (Cont'd)

BmrI
BsiEI
StyI
PpuMI
 ACTGGGGTTTCAGATACAATAATGCGAACCATGCCCGGTCGGGTTTTTTTCAAAATGTCCTAGCGGTTTGAAAAGTGTTCATCCTTGGACAAGCTGCGAGACTAAGGA
 136 138 140 142 144 146 148 150 152 154 156 158 160 162 164 166 168 170
 >T>G>V>S>D>T>I>C>E>P>C>P>V>G>F>S>N>V>S>S>A>F>E>K>C>H>P>W>T>S>C>E>T>K>D>



Tth111I
BSU36I
AlwI
BsiHKAI
PflFI
 CCTCGTCGTTCAGCAAGCGGGTACGAATAAACTGATGTTGTTGTGGGCTCAGGATCGACTTAGAGCACTGGTTGTGCATCCCTATTATATTGGGATTTTGTTCG
 172 174 176 178 180 182 184 186 188 190 192 194 196 198 200 202 204 206
 >>L>V>V>Q>Q>A>G>T>N>K>T>D>V>V>C>G>P>Q>D>R>L>R>A>L>V>V>I>P>I>I>F>G>I>L>F>



FIG. 10E (Cont'd)

Construct 5 - full CD40 no scFv (1692 bp) (from 643-1177 bp)

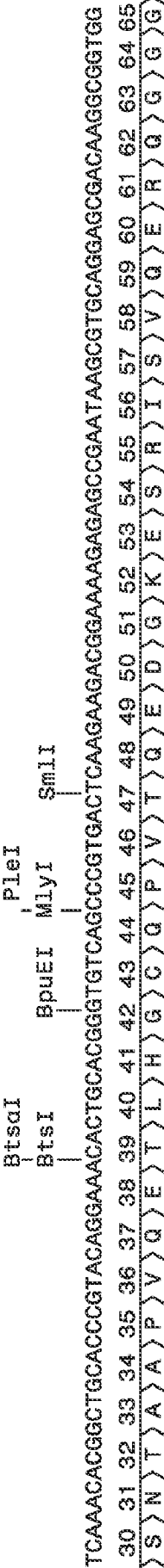
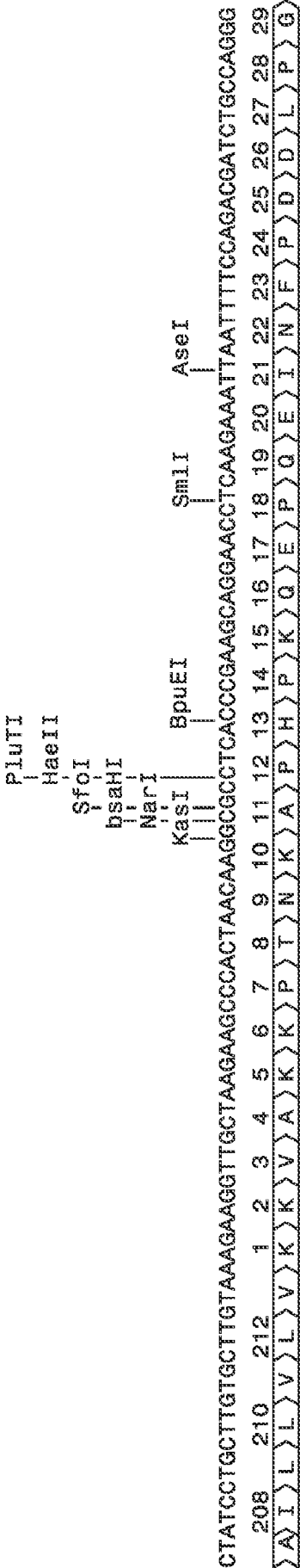


FIG. 10E (Cont'd)

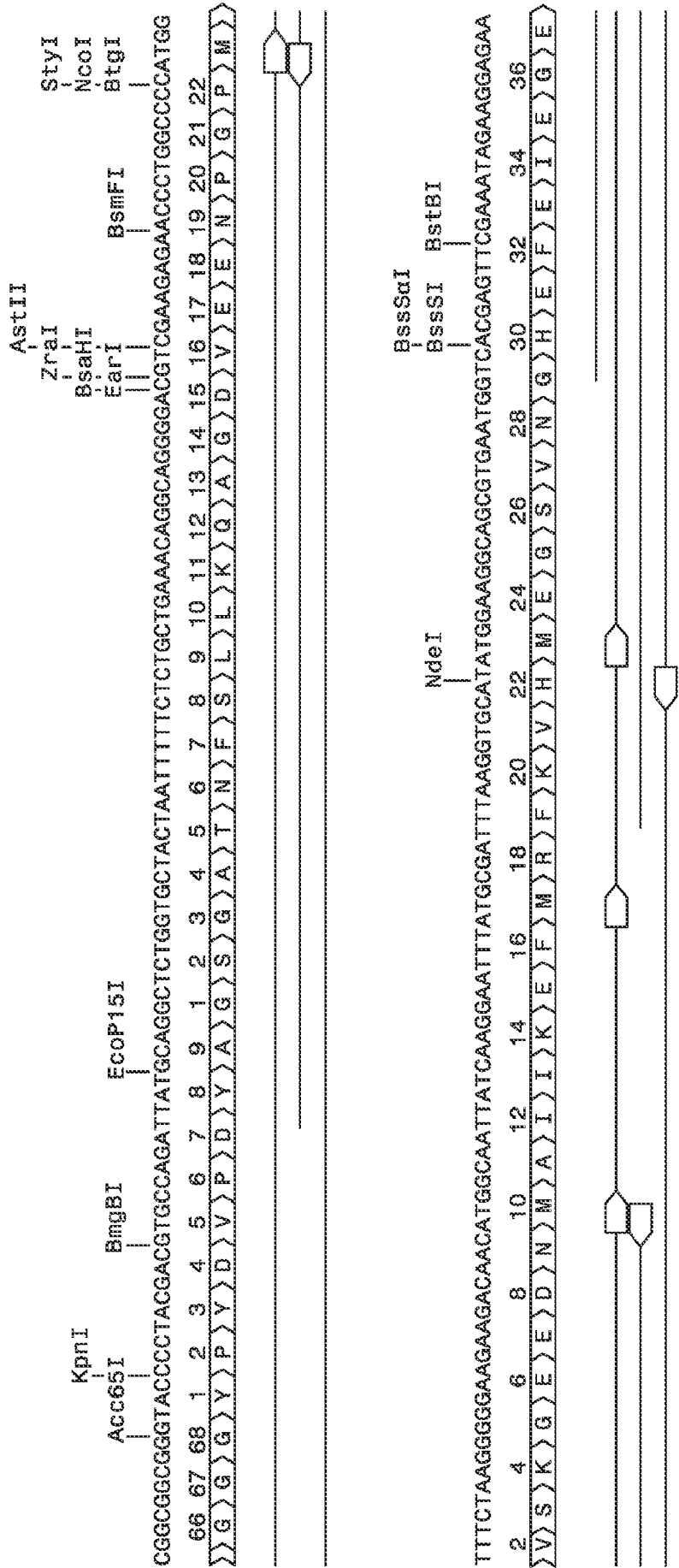


FIG. 10E (Cont'd)

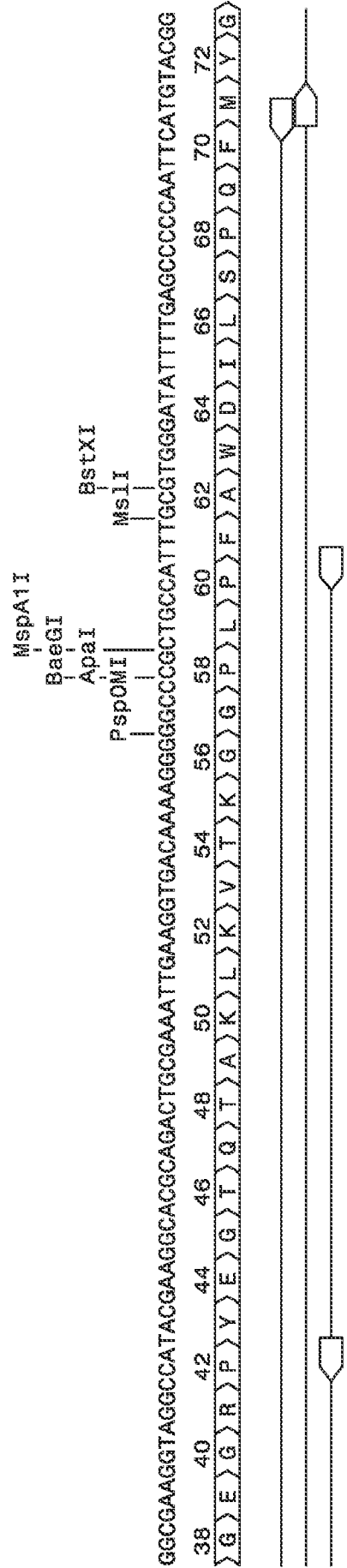


FIG. 10E (Cont'd)

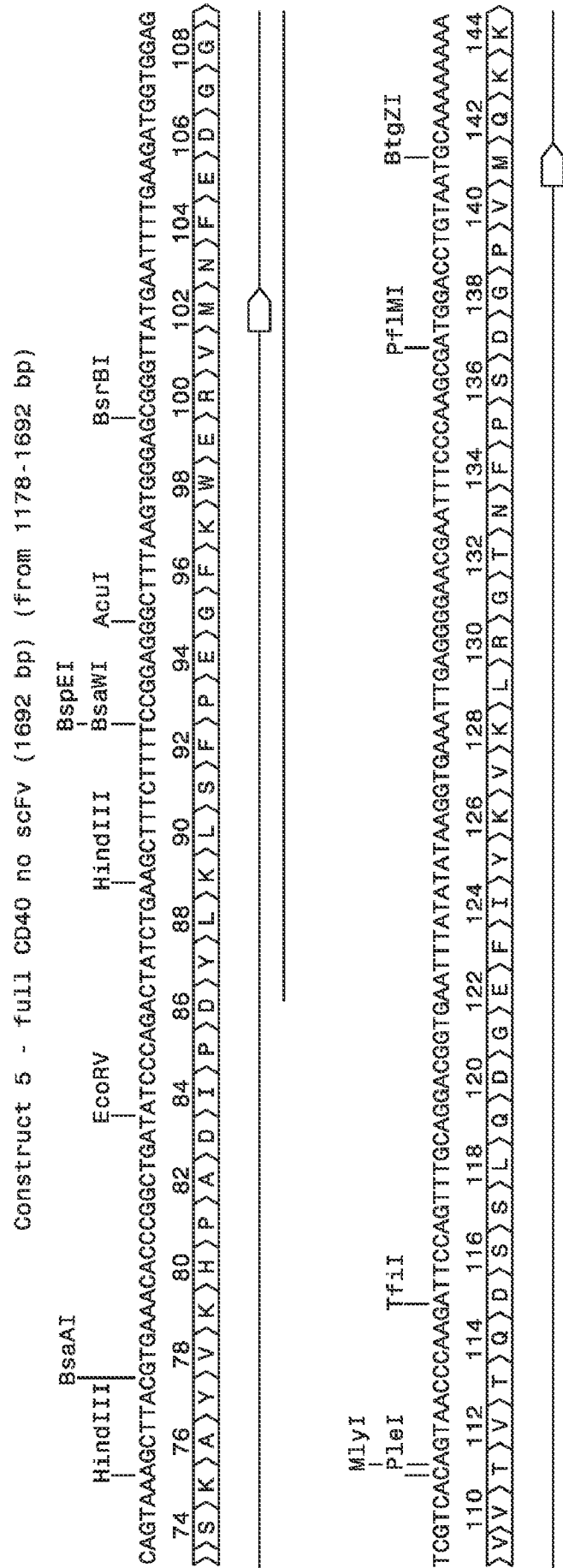


FIG. 10E (Cont'd)

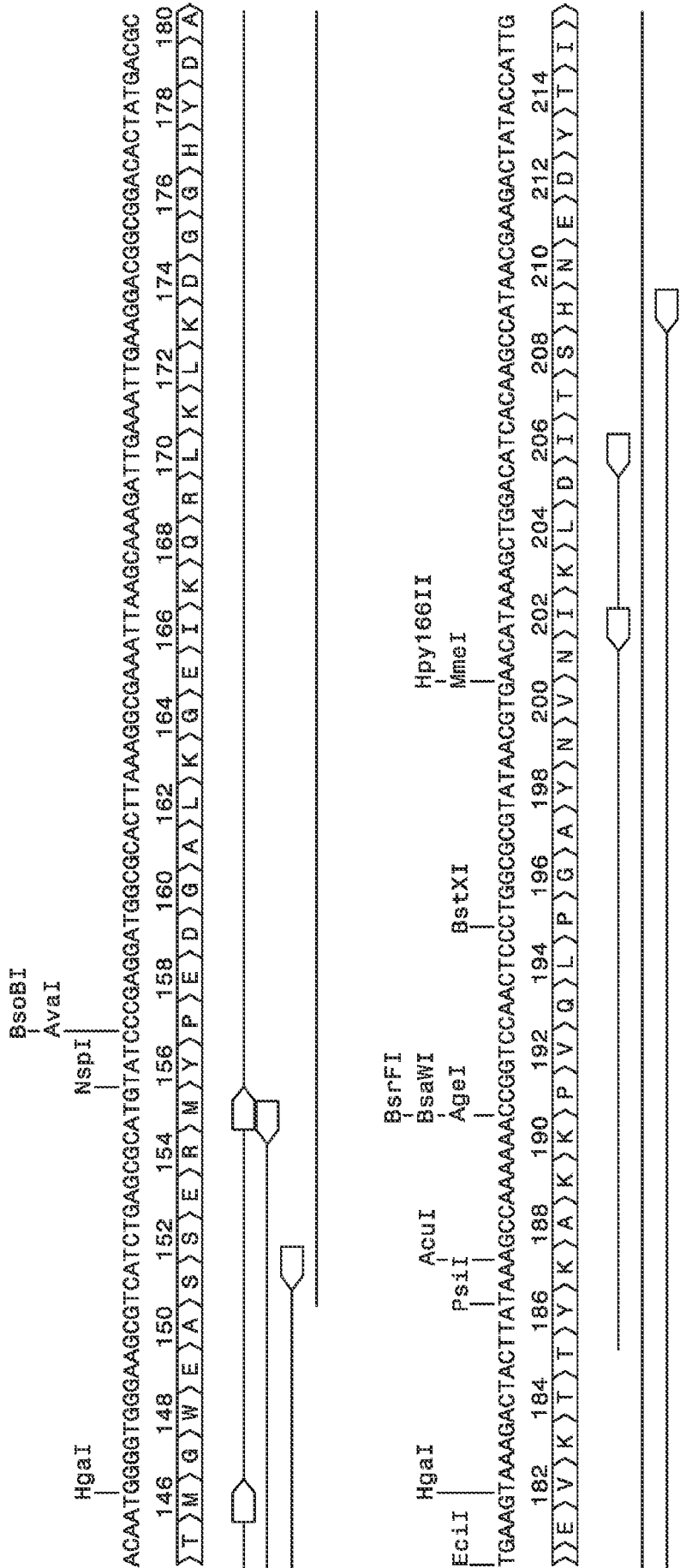


FIG. 10E (Cont'd)

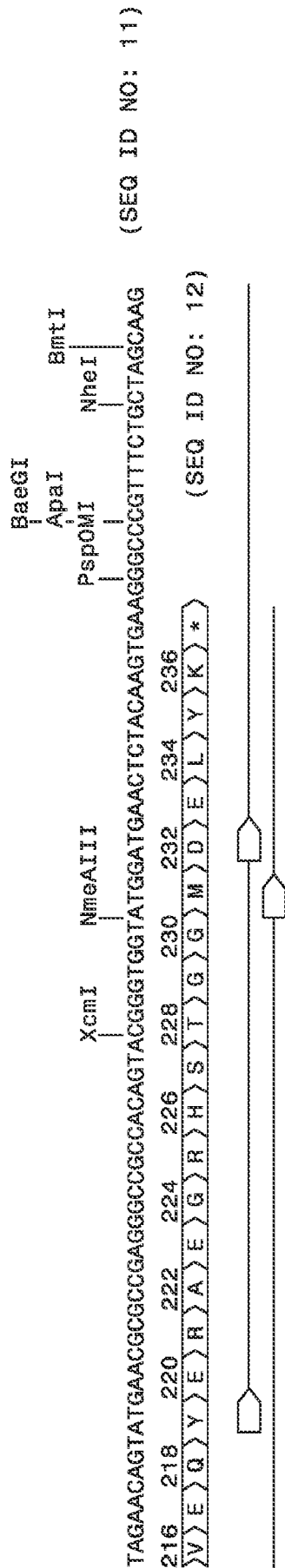


FIG. 10E (Cont'd)



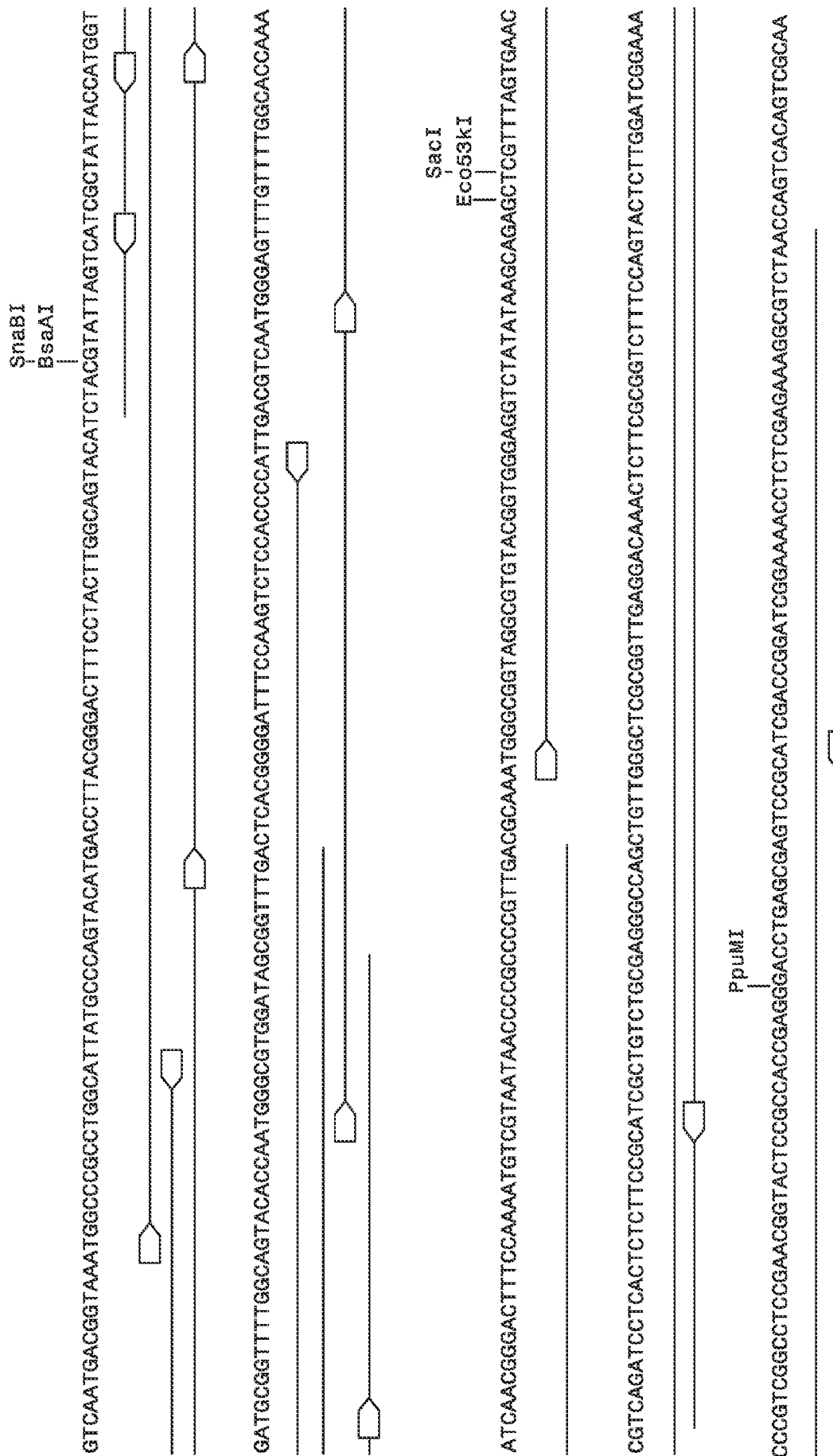


FIG. 10F (Cont'd)

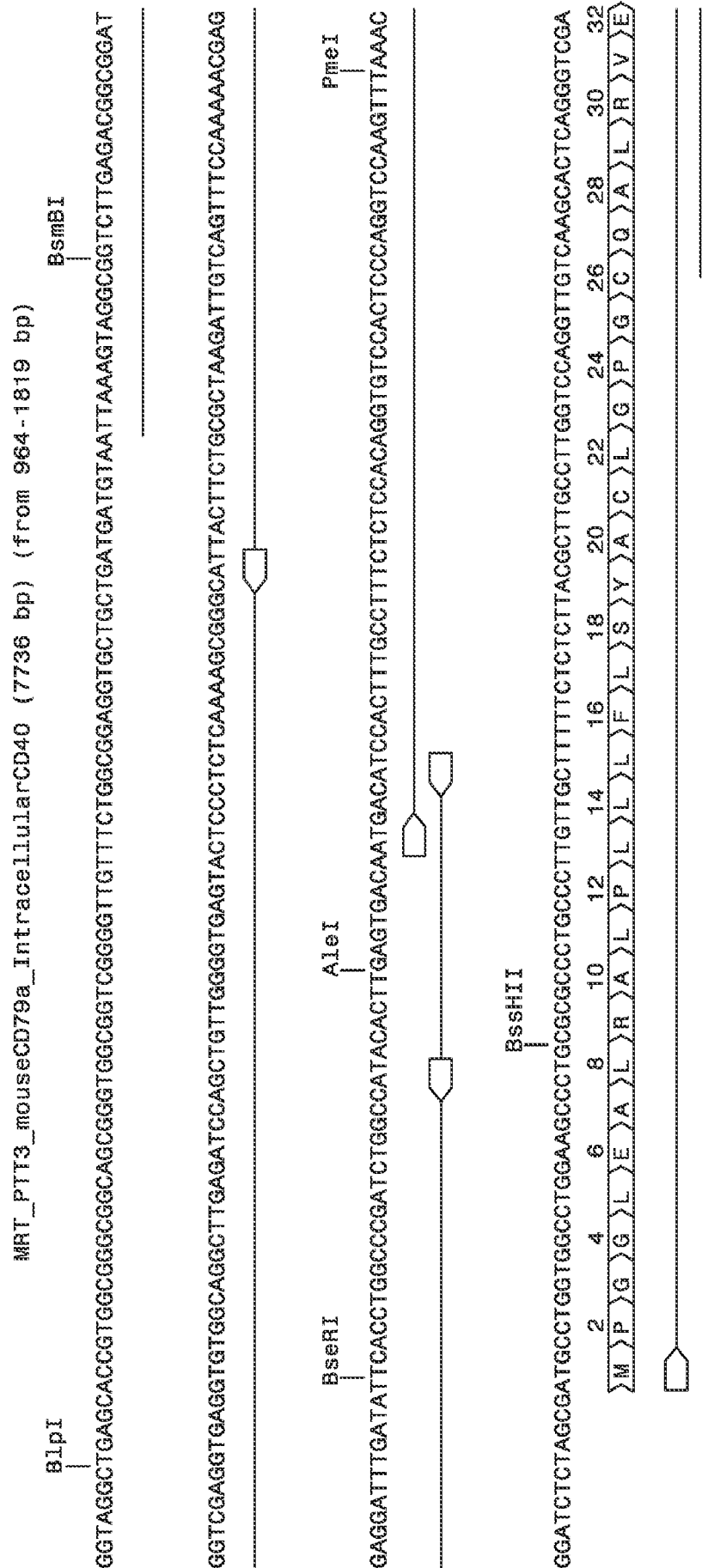


FIG 10F (Contd)

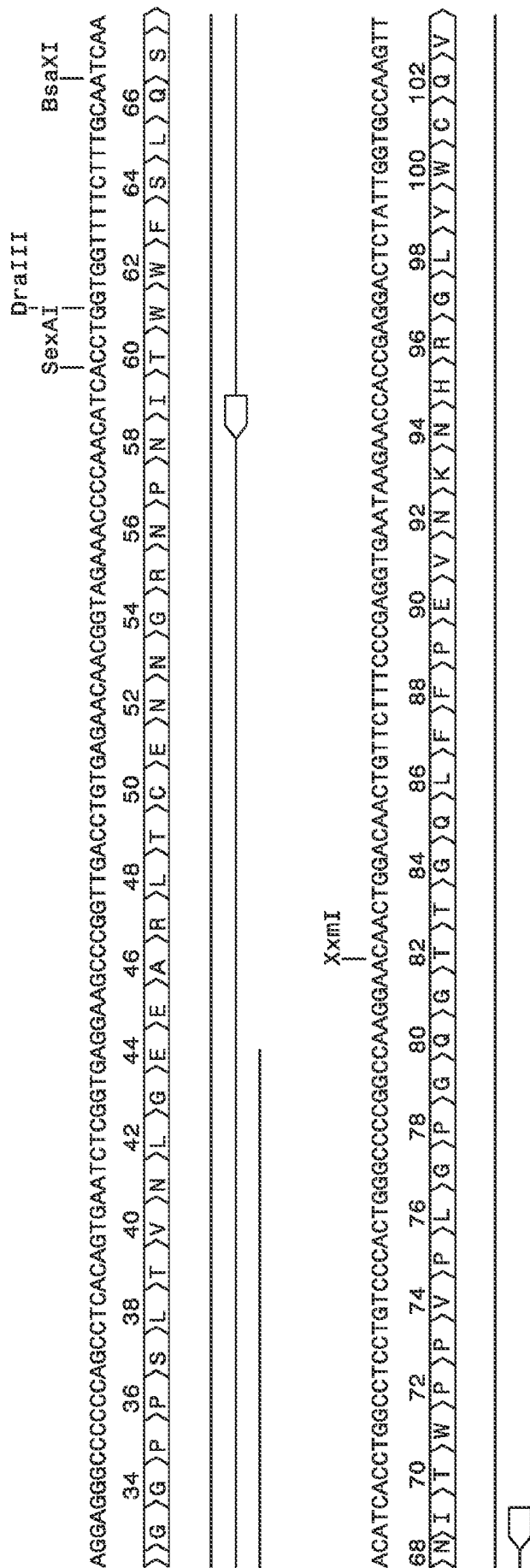


FIG. 10F (Cont'd)

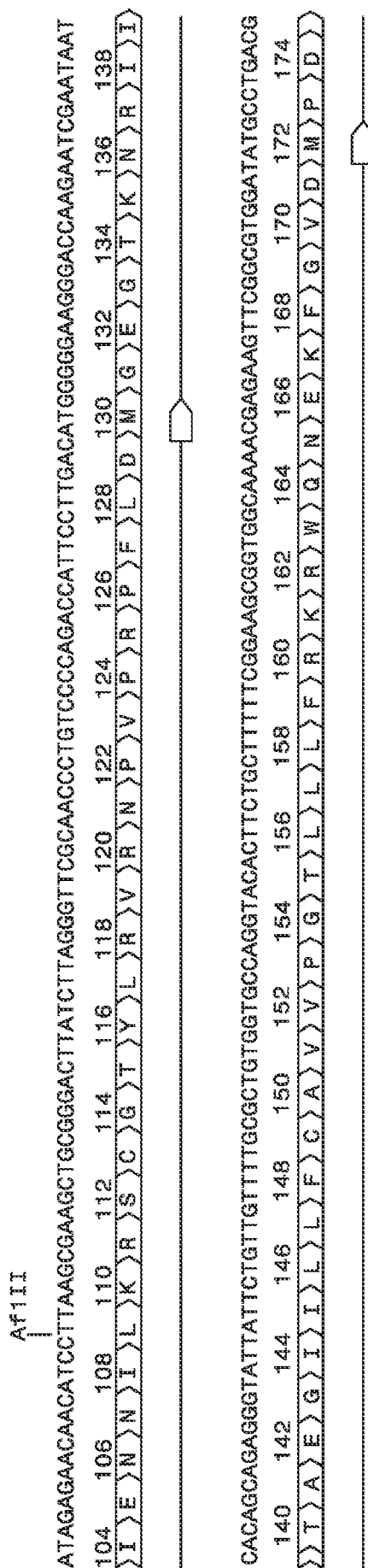


FIG. 10F (Cont'd)

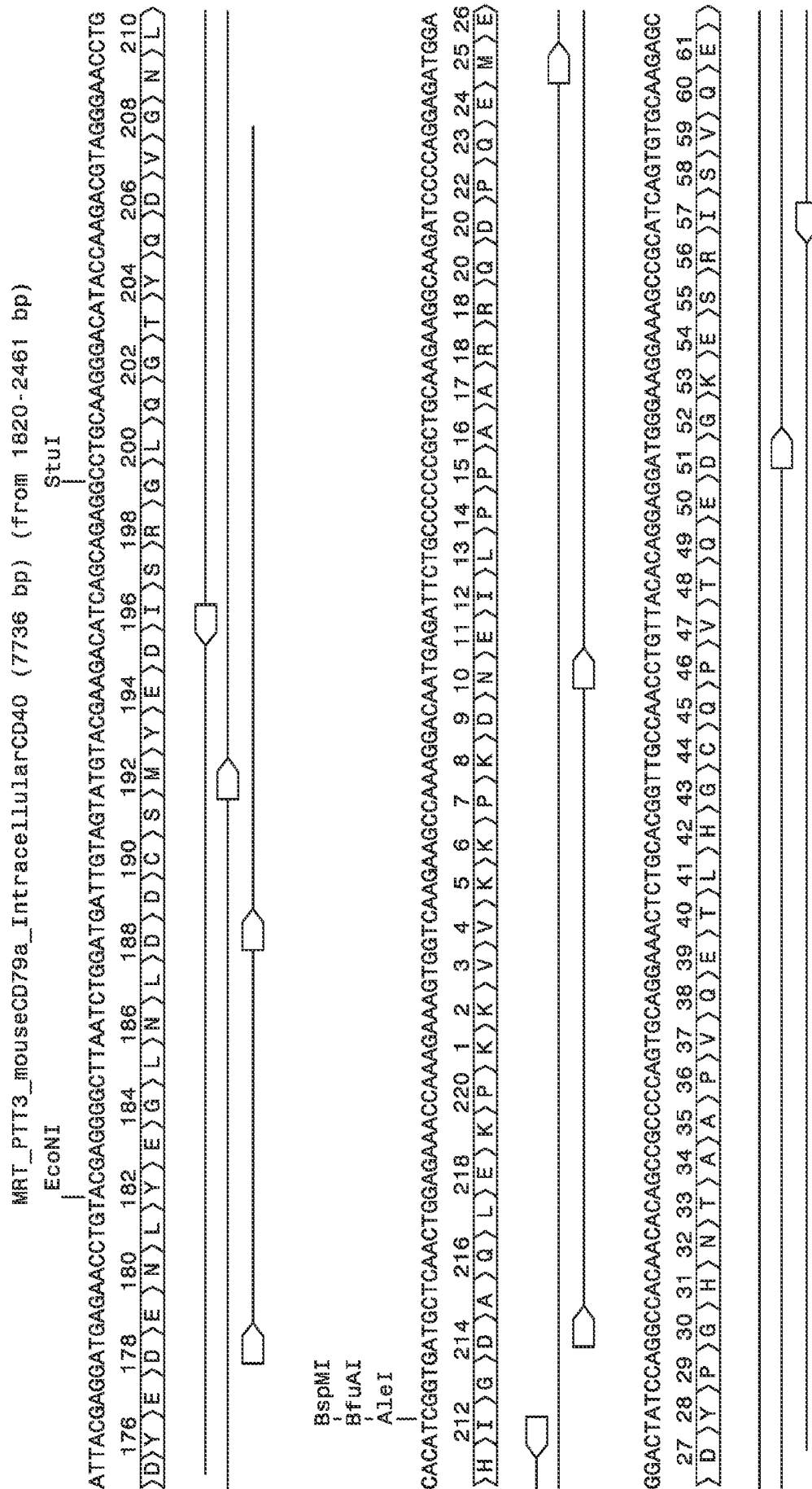


FIG. 10F (Cont'd)

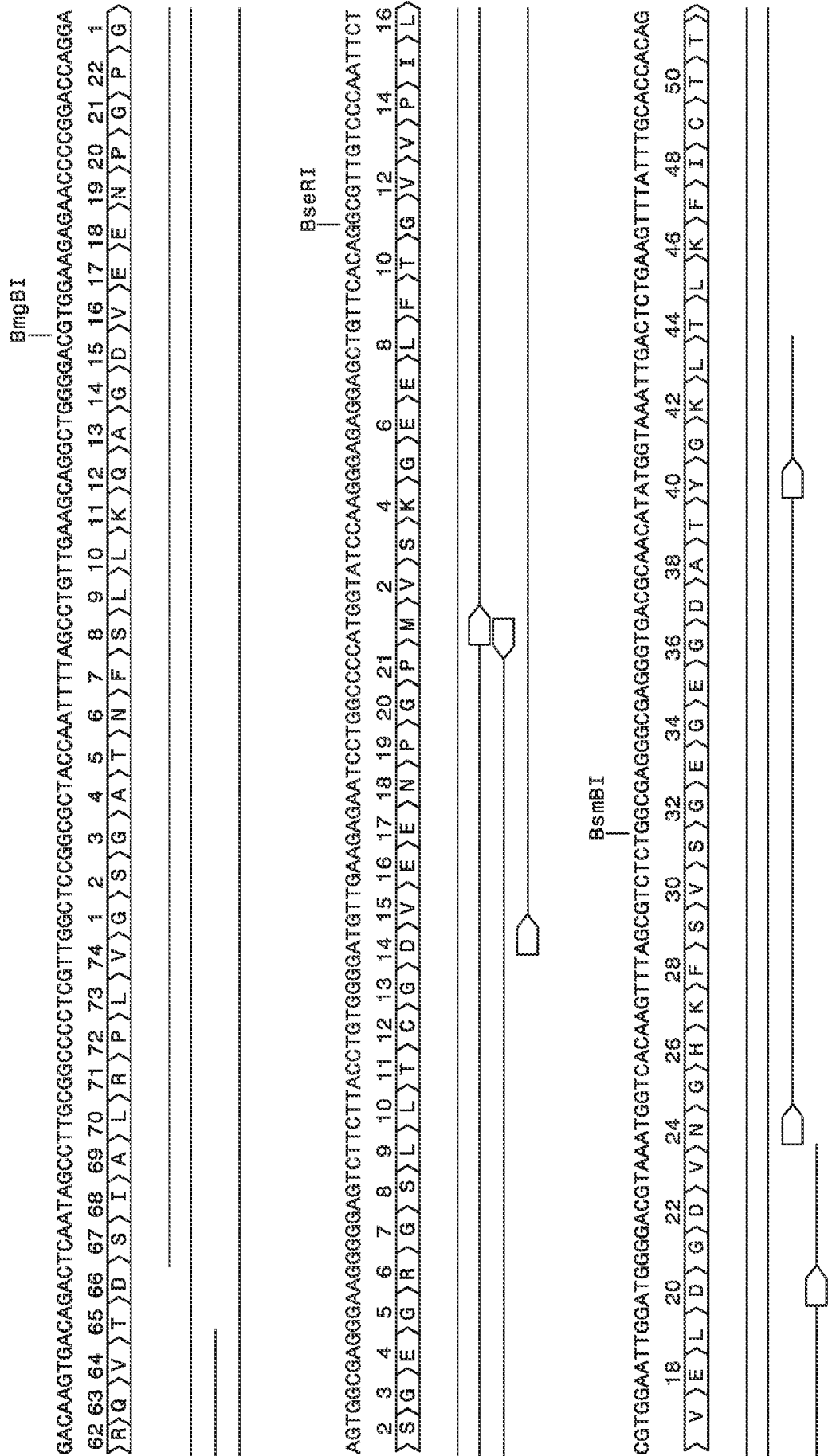
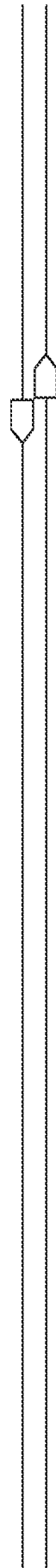


FIG. 10F (Cont'd)

MRT_PTT3_mouseCD79a_IntracellularCD40 (7736 bp) (from 2462-3210 bp)

GTAACTTCCTGTGGCCTACACTTGTCACTACTCTGACCTATGGCGTGCAATGTTTCTCCGCTACCCCTGACCATATGAAACAACACACGATTTTTTTAAATCT
 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86
 >G>K>L>P>V>P>W>P>T>L>V>T>T>L>T>Y>G>V>Q>C>F>S>R>V>P>D>H>M>K>Q>H>D>F>F>K>S>



BbvCI

PspXI

GCCATGCCCGAAGGGTATGTCAGGAACGGACTATTTCTTTAAGGACGACGGCAATTATAAACTCGAGCTGAGGTCAAATTTGAAGGTGACACCTTGGTCAACAG
 88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118 120 122
 >A>M>P>E>G>Y>V>Q>E>R>T>I>F>F>K>D>D>G>N>Y>K>T>R>A>E>V>K>F>E>G>D>T>L>V>N>R>



FIG. 10F (Cont'd)

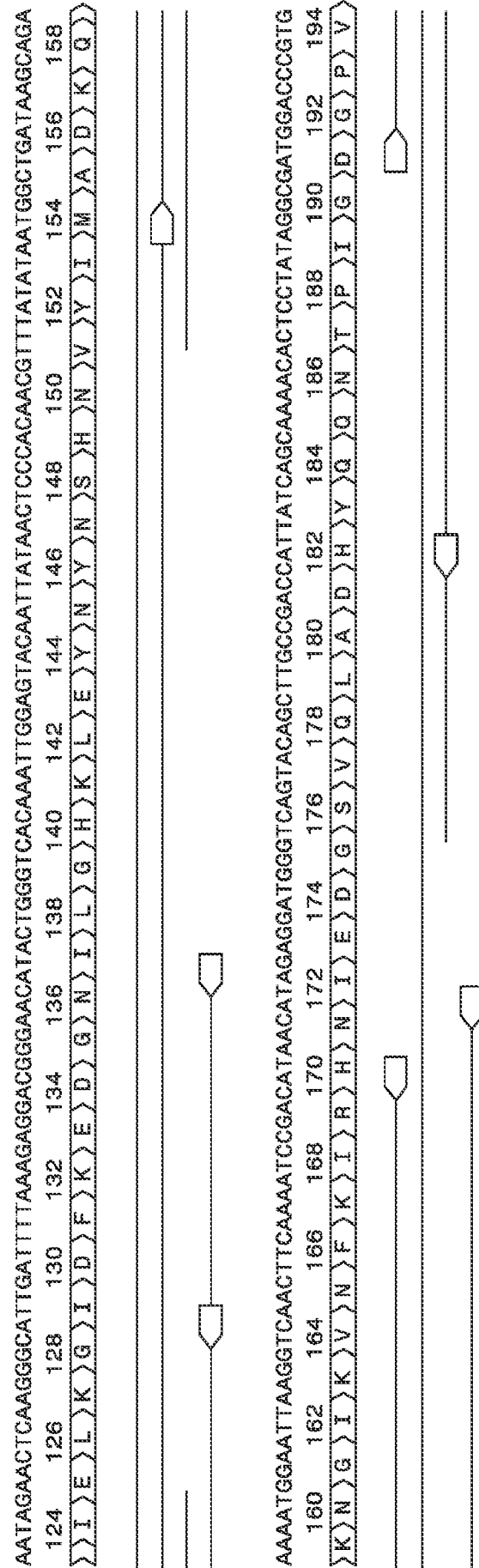


FIG. 10F (Cont'd)

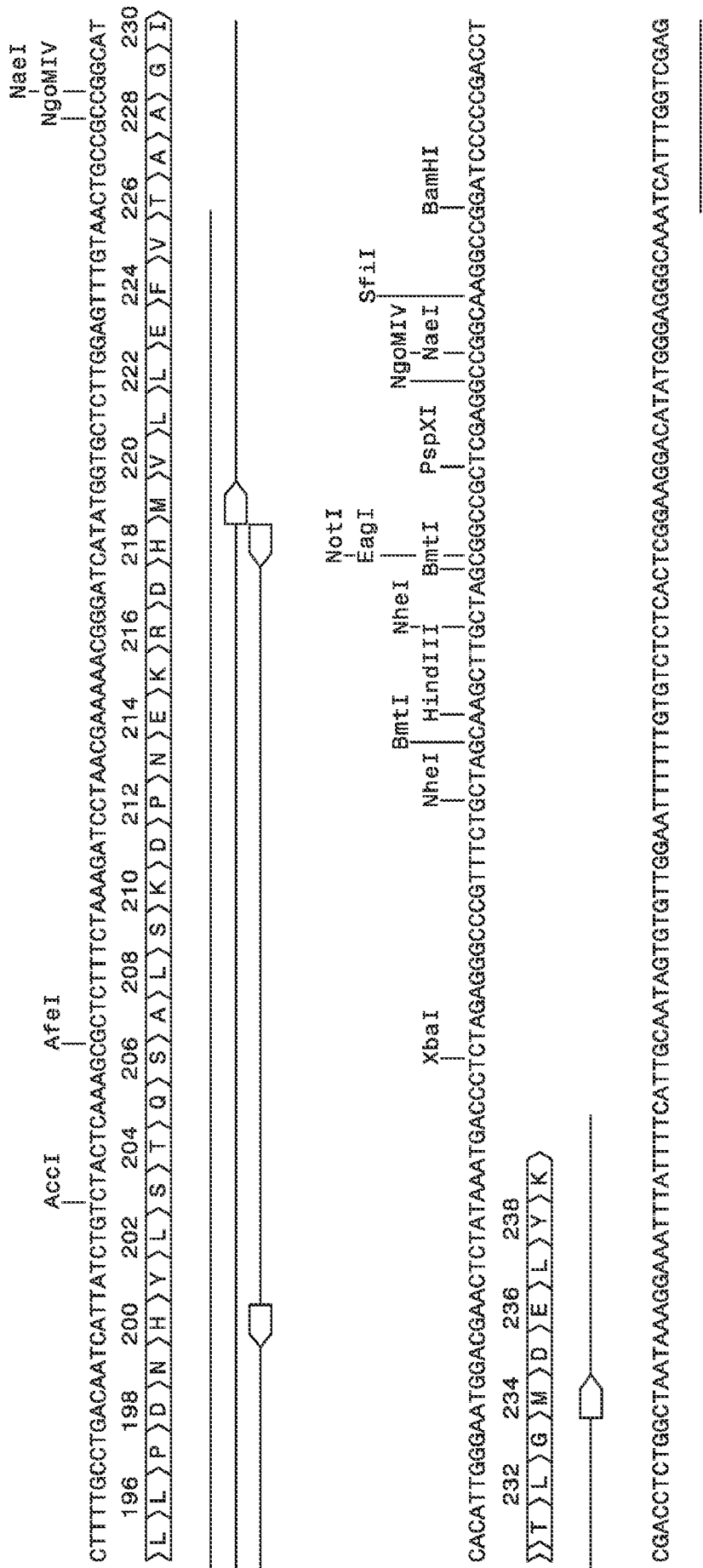


FIG. 10F (Cont'd)

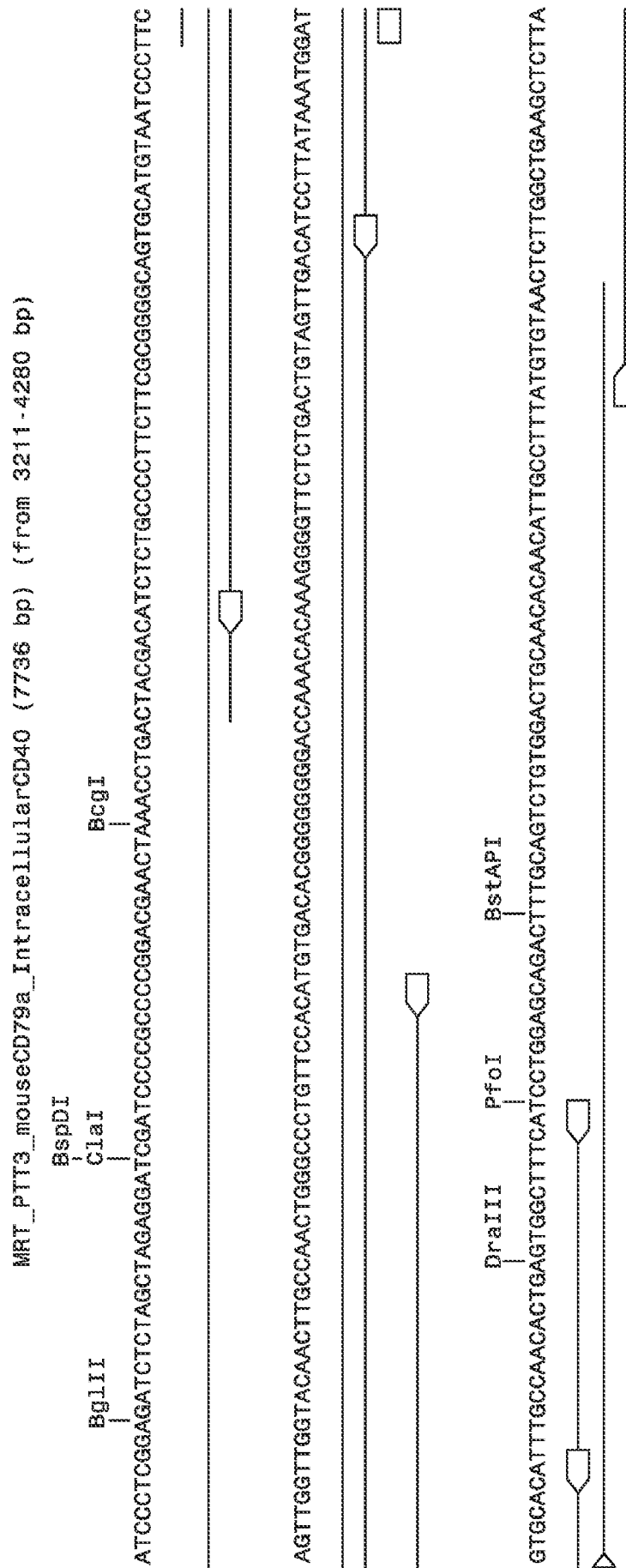


FIG. 10F (Cont'd)

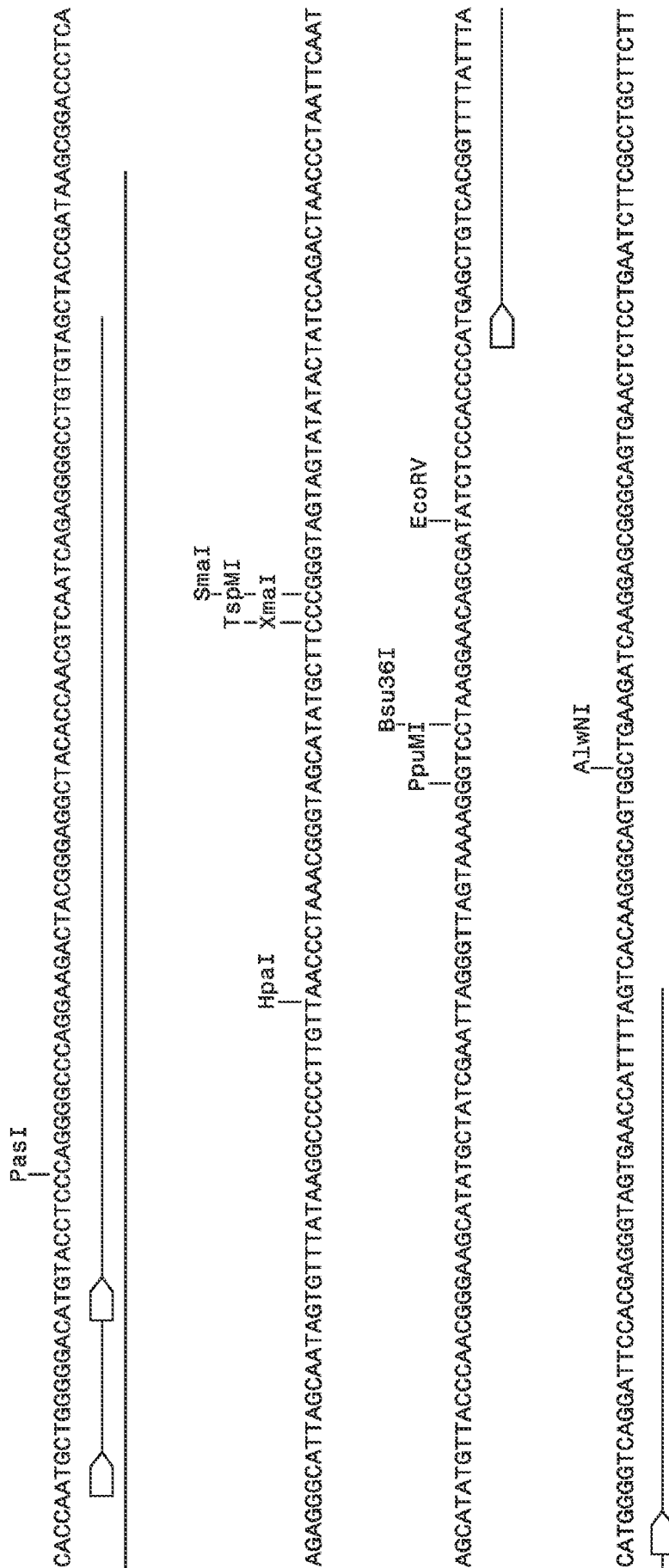


FIG. 10F (Cont'd)



FIG. 10F (Cont'd)

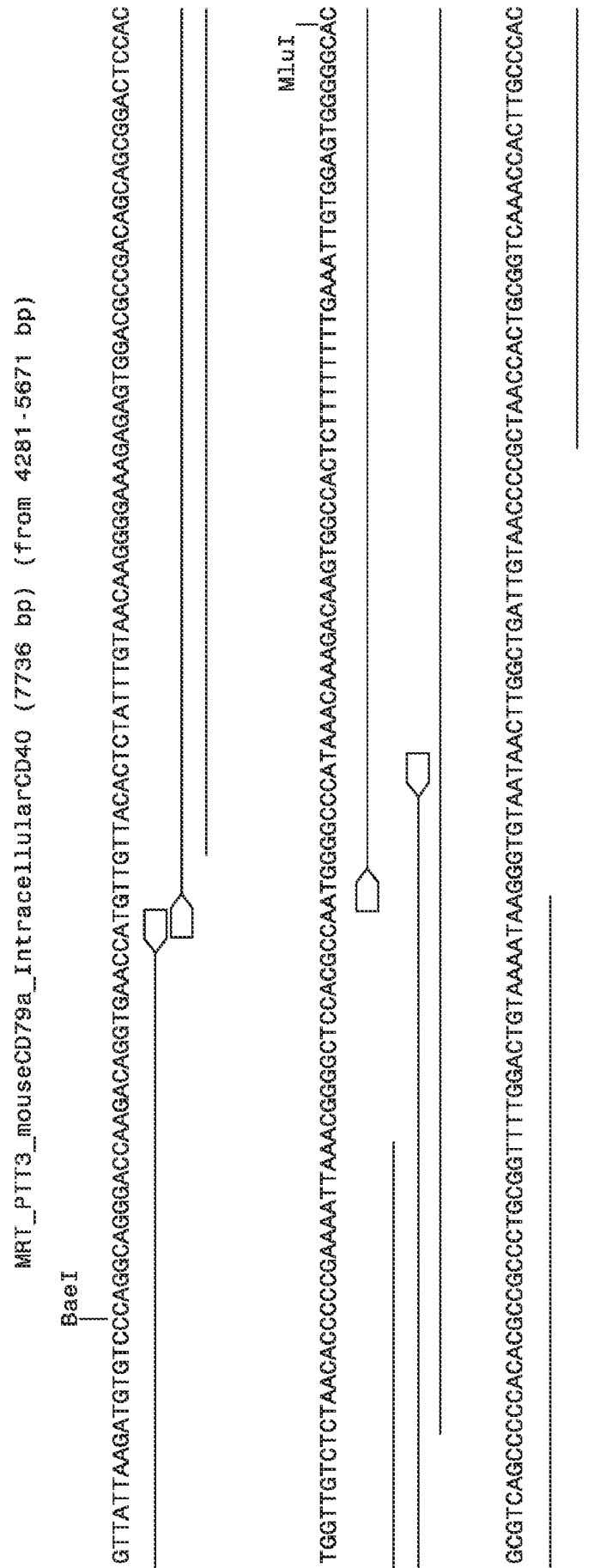


FIG. 10F (Cont'd)

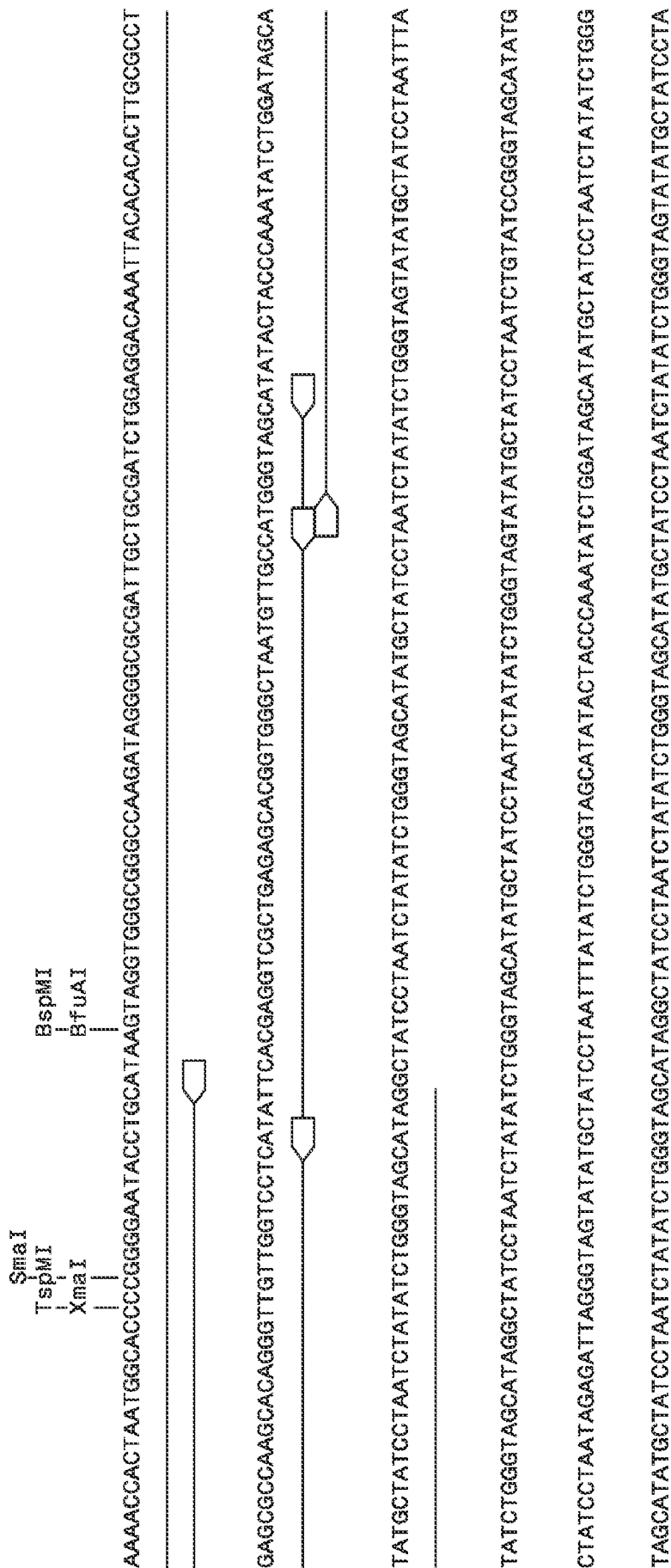


FIG. 10F (Cont'd)

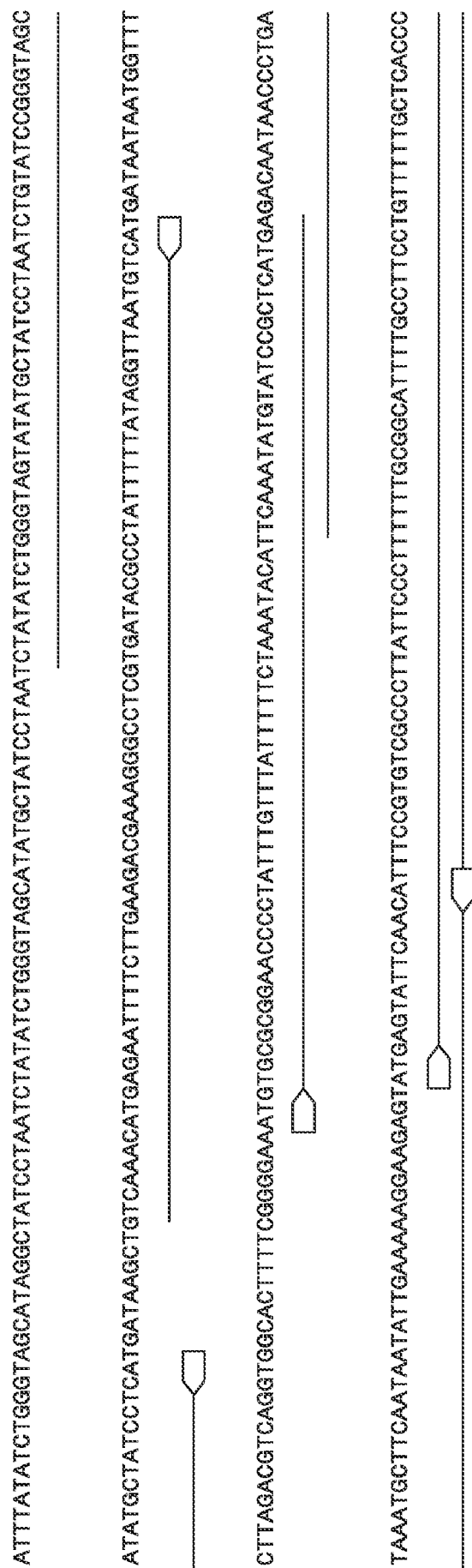


FIG. 10F (Cont'd)

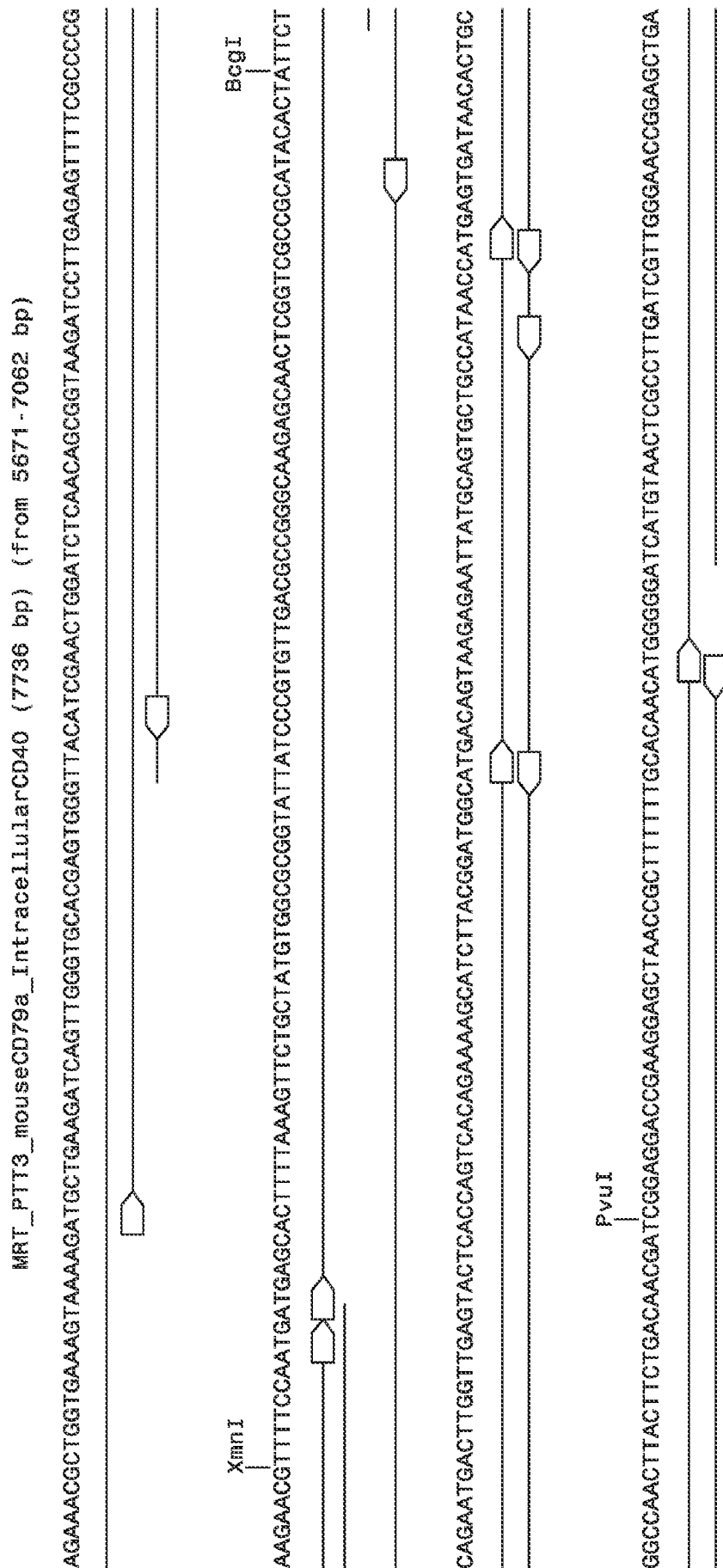


FIG. 10F (Cont'd)

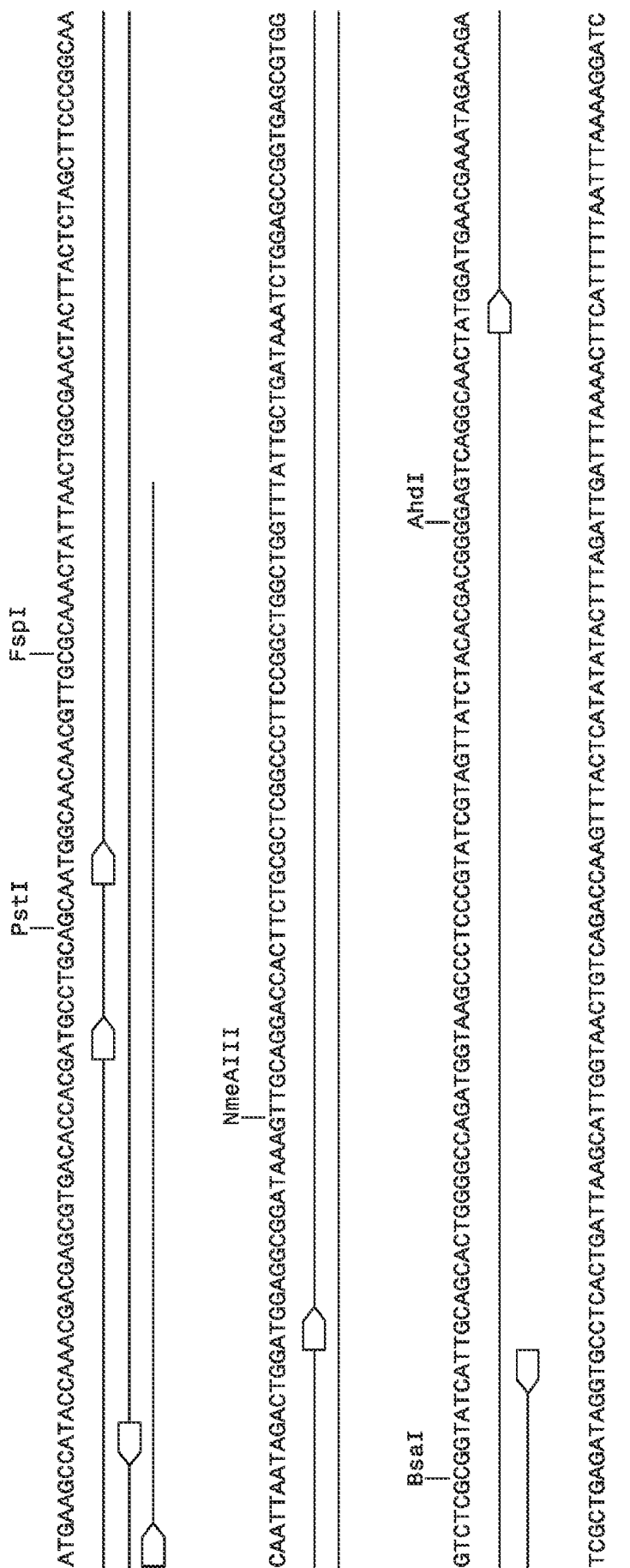


FIG. 10F (Cont'd)

TAGGTGAAGATCCTTTTGTGATAATCTCATGACCACAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGA
TCCTTTTTTCTGGCGGTAATCTGCTGCTTGGCAACAAAAAACACCGGTACCAGCGGTGGTTTTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAA
CTGGCTTCAGCAGAGCGCAGATACCAAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTTCAAGAAGTCTGTAGCACCGCCTACATACCTCGCTCTGCTA
ATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGGCAGCGGTCGGGCTGAACGGGGGG
TTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGGCCACGCTTCCCAGAGGGAGAAAGGCGGACA

ALWNI
└



FIG. 10F (Cont'd)

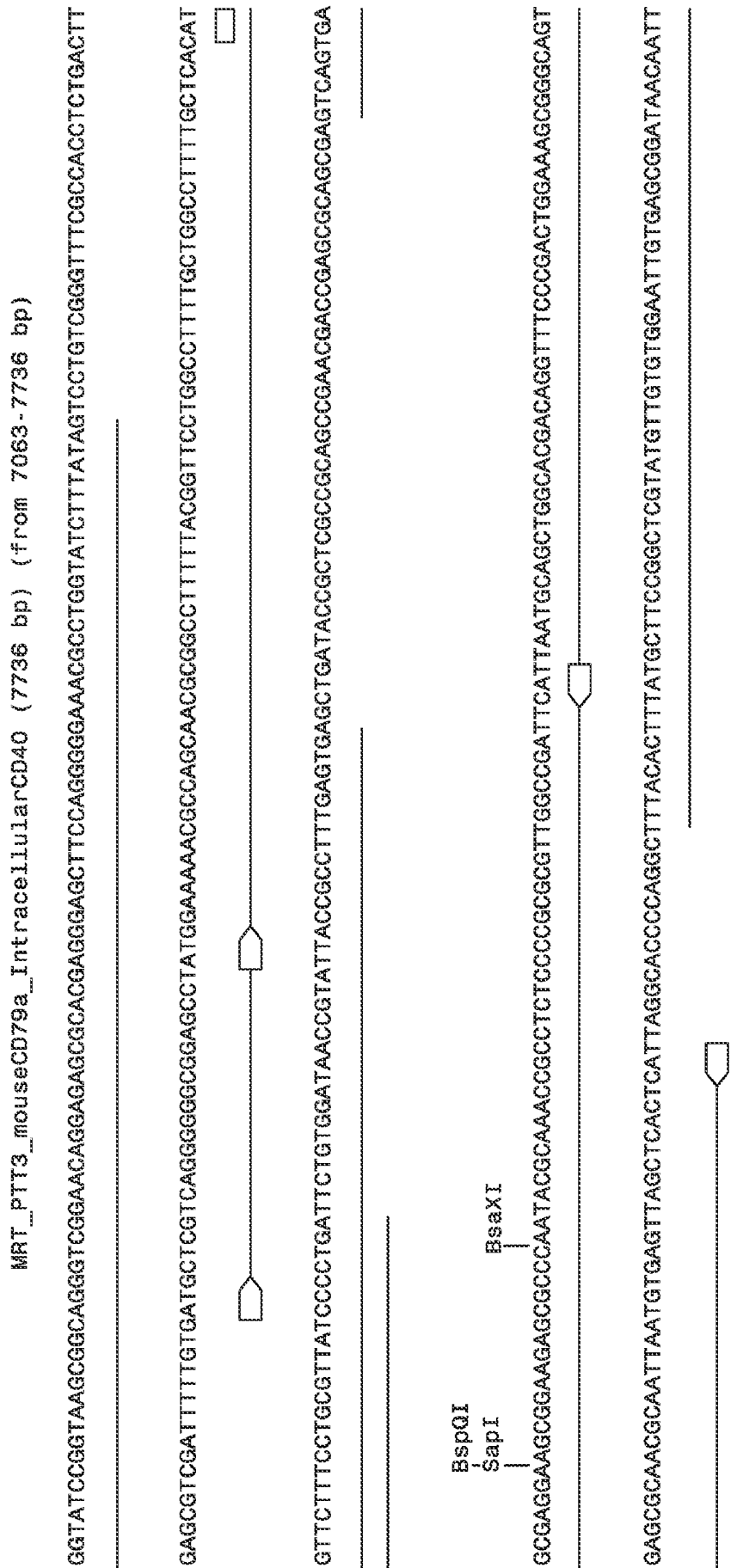


FIG. 10F (Cont'd)

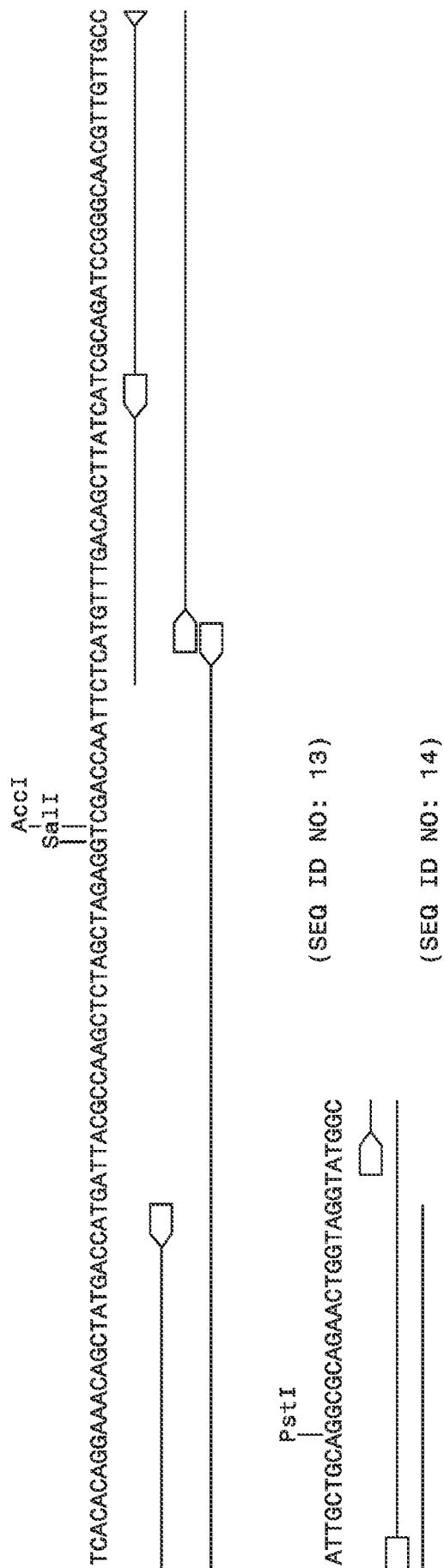


FIG. 10F (Cont'd)

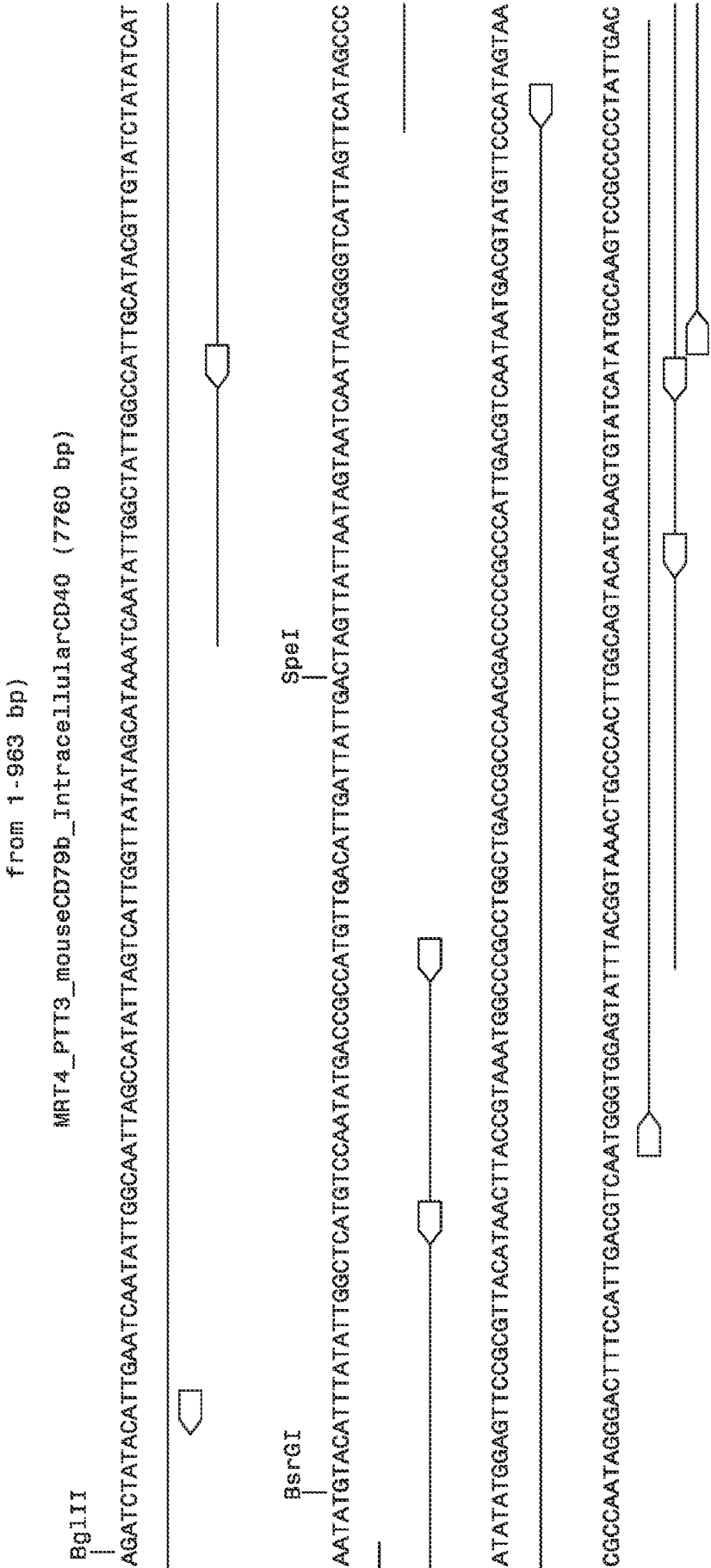


FIG. 10G

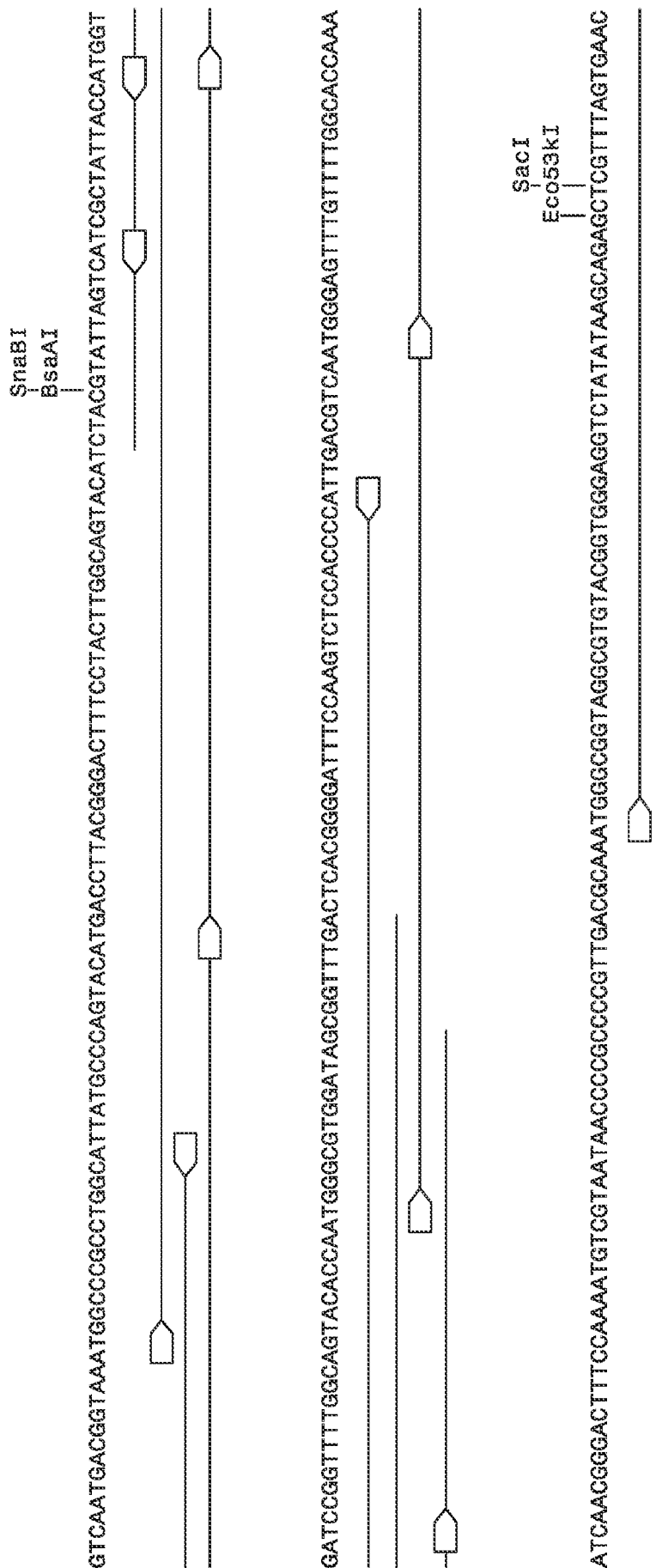


FIG. 10G (Cont'd)

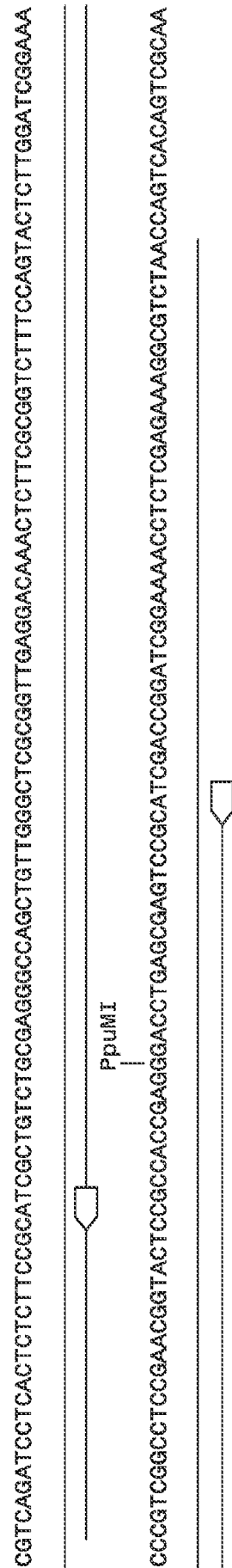


FIG. 10G (Cont'd)

MRI4_PIT3_mouseCD79b_IntracellularCD40 (7760 bp) (from 964-1819 bp)

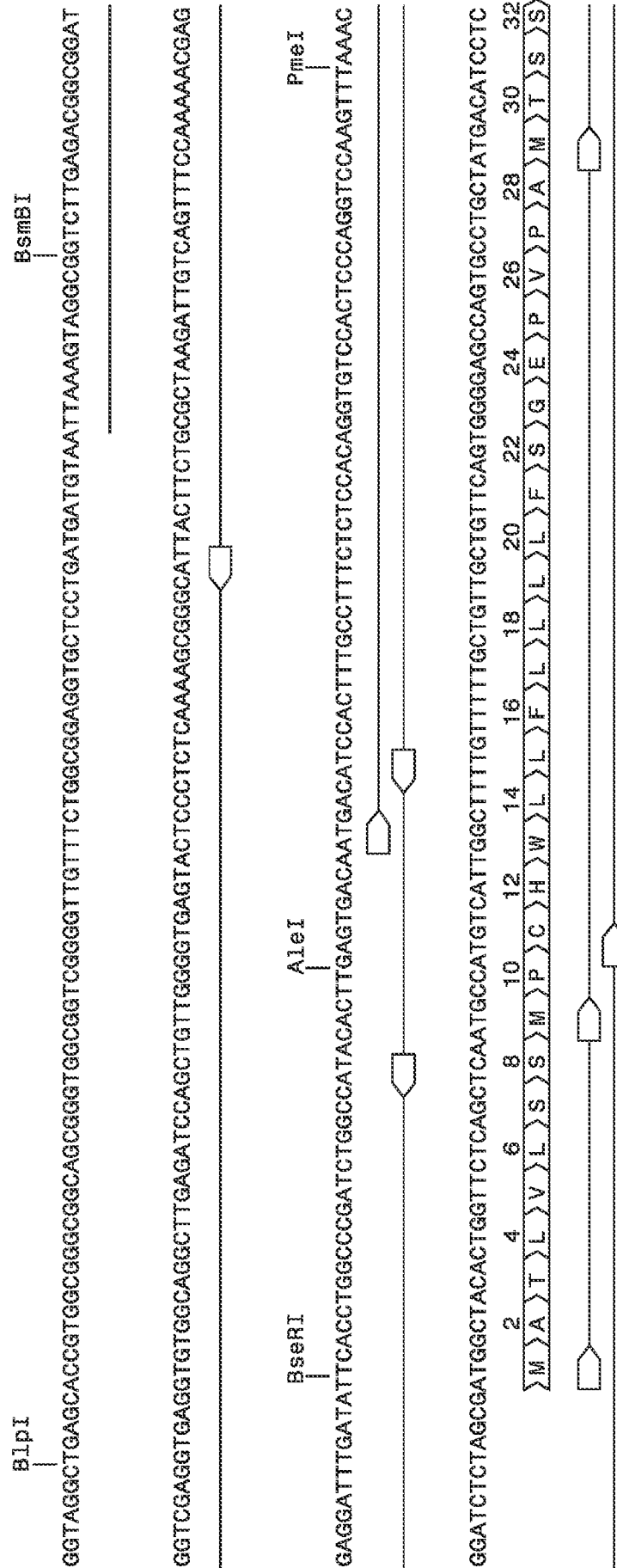


FIG. 10G (Cont'd)

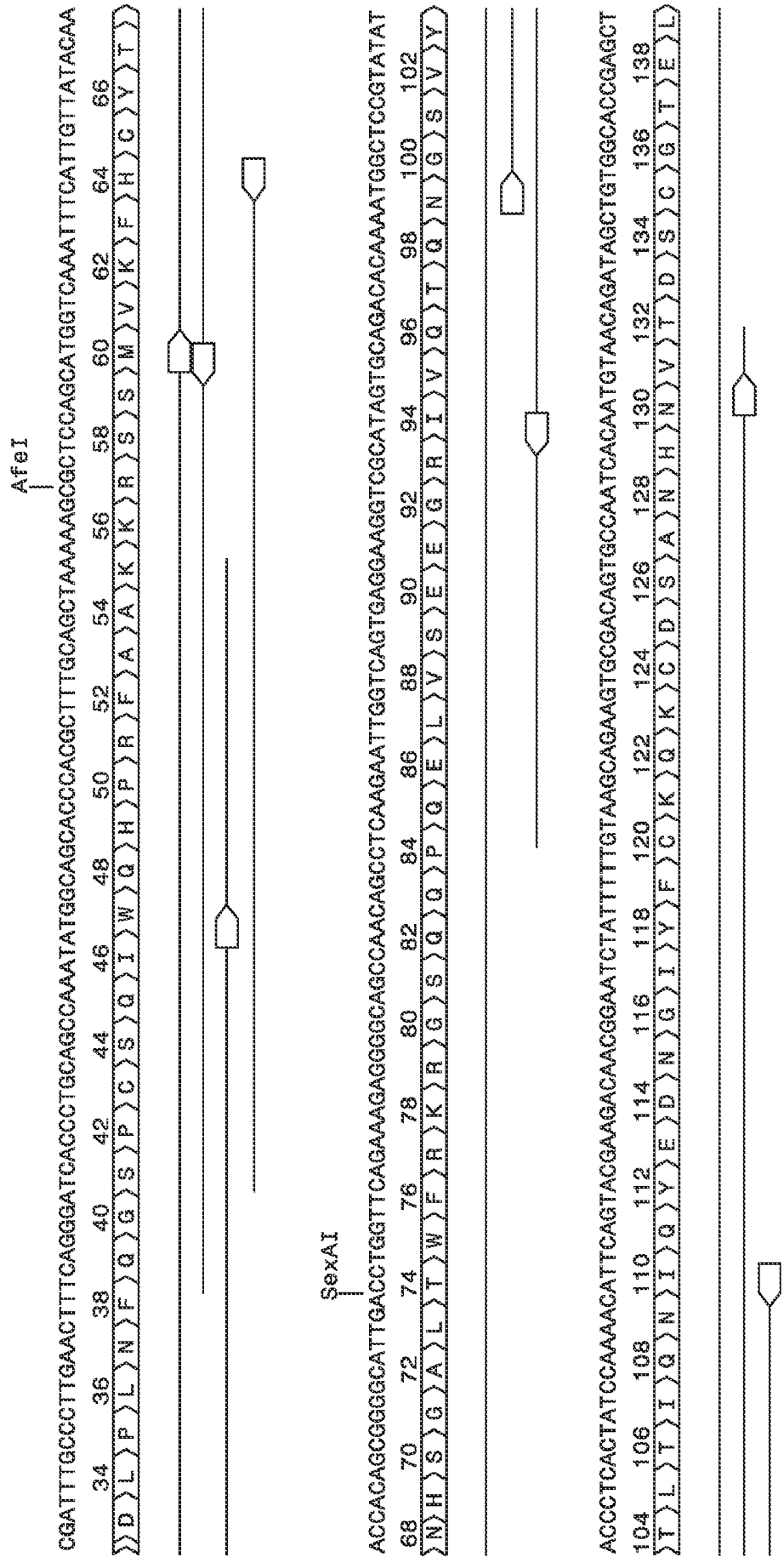


FIG. 10G (Cont'd)

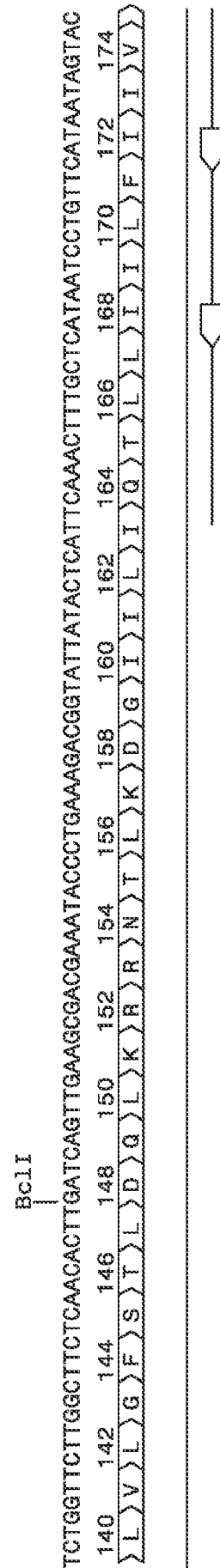


FIG. 10G (Cont'd)

MRI5_PTT3_mouseCD79b__IntracellularCD40 (7760 bp) (from 1820-2568 bp)

ECONI

CTATCTTCCTGCTCCTTGATAAGGACGACGGGAAAGCAGGCATGGAAGAAGATCACACCTACGAGGGTTGAATATCGACCAGACAGCCACCTACGAAGACATTGTG
176 178 180 182 184 186 188 190 192 194 196 198 200 202 204 206 208 210
>P>I>F>L>L>L>L>D>K>D>D>G>K>A>G>M>E>E>D>H>T>Y>E>G>L>N>I>D>Q>T>A>T>Y>E>D>I>V>

ACTCTCCGAACCGGGAGGTGAAATGGAGTGTAGGAGAACACCCCGGTCAAGAAAGAAAGTGGTCAAGAAAGCCAAAGGACAATGAGATTCTGCCCCCGCTGCAAG
212 214 216 218 220 222 224 226 228 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
>T>L>R>T>G>E>V>K>W>S>V>G>E>H>P>G>Q>E>K>K>V>V>K>K>P>K>D>N>E>I>L>P>P>A>A>R>

AAGGCAAGATCCCCAGGAGATGGAGGACTATCCAGGCCCAACACACAGCCGCCCCAGTGCAGGAAACTCTGCACGGTTGCCAACCTGTTACACAGGAGGATGGGAAGG
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53
>R>Q>D>P>Q>E>M>E>D>Y>P>G>H>N>T>A>A>P>V>Q>E>T>L>H>G>C>Q>P>V>T>Q>E>D>G>K>

FIG. 10G (Cont'd)

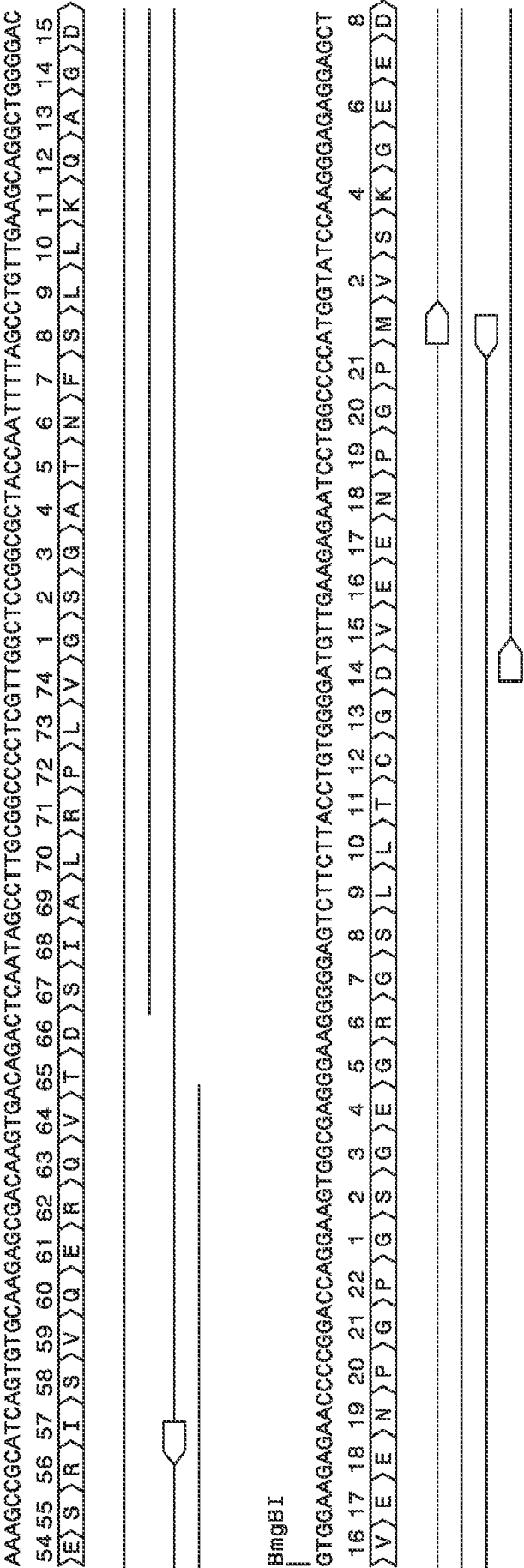


FIG. 10G (Cont'd)

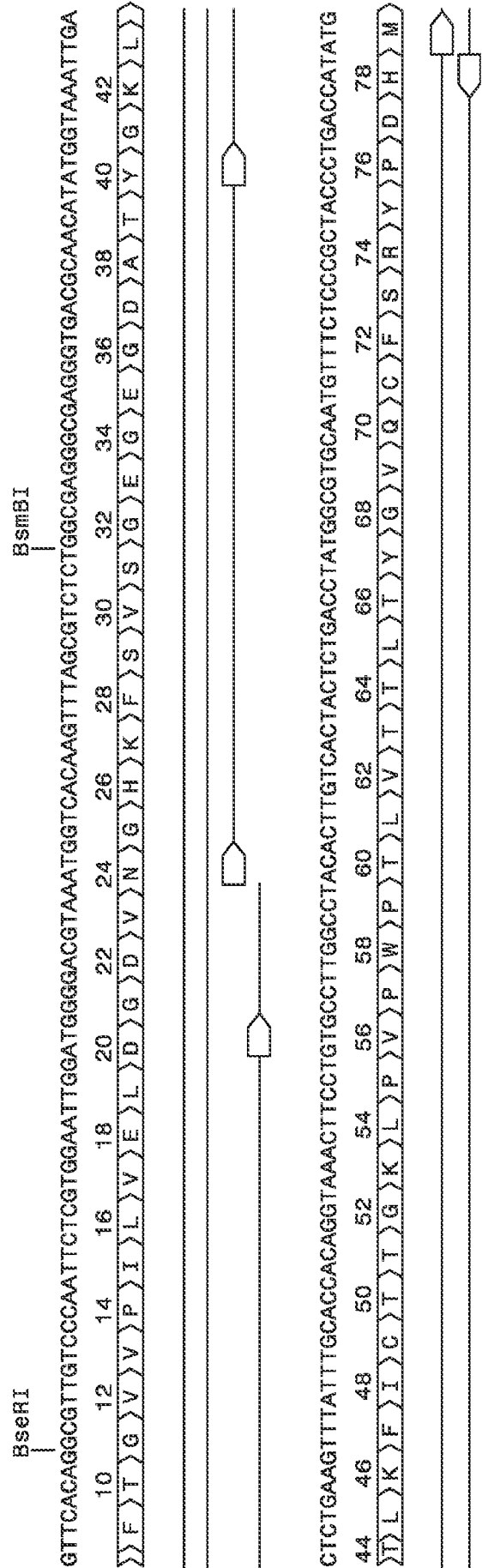


FIG. 10G (Cont'd)

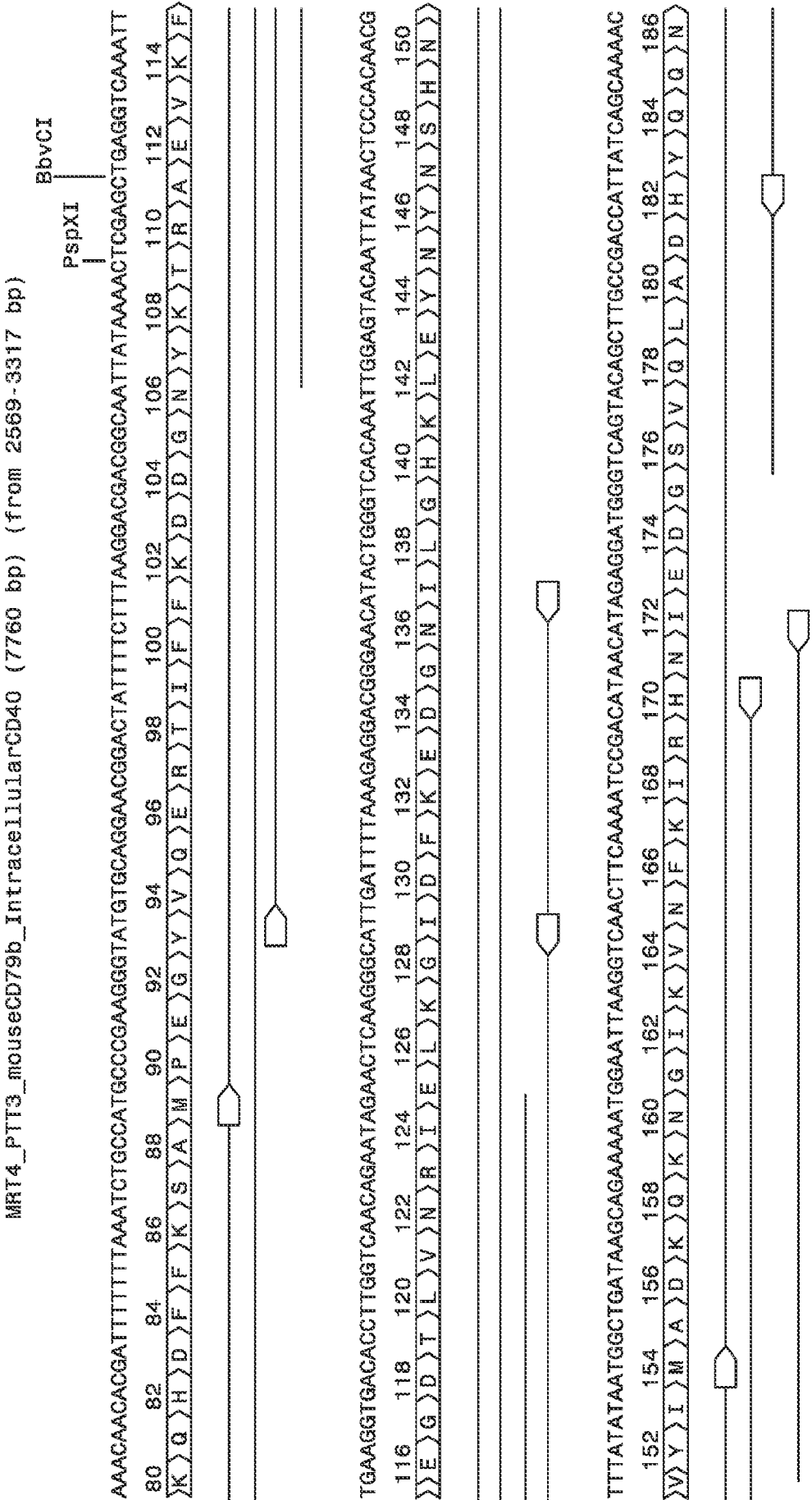


FIG. 10G (Cont'd)

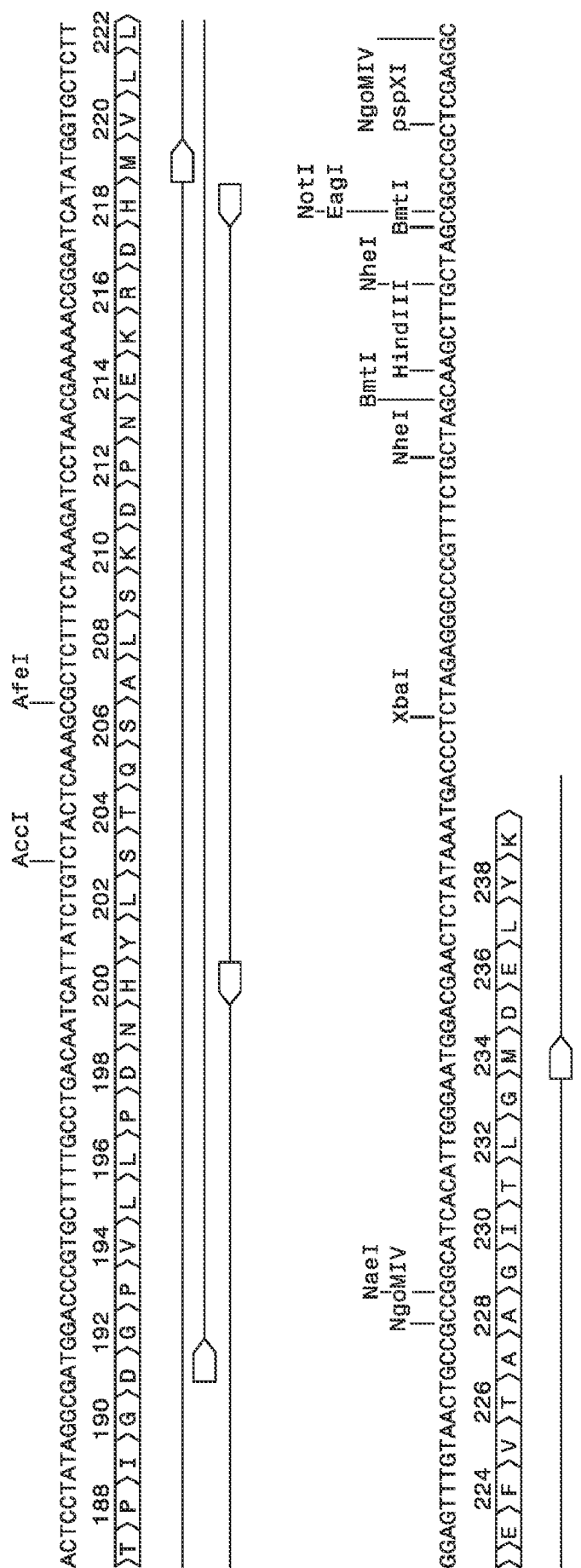


FIG. 10G (Cont'd)

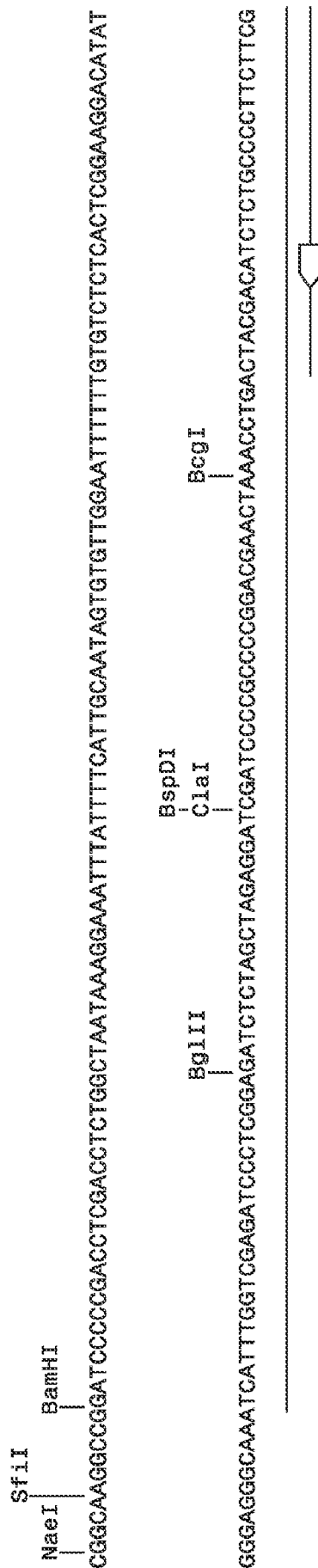


FIG. 10G (Cont'd)

MRT4_PTT3_mouseCD79b__IntracellularCD40 (7760 bp) (from 3318-4387 bp)

CGGGGCGAGTGCATGTATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCACATGTGACACGGGGGGGACCAACACAAAGGGGTTCTCTGACTG

DraIII	pfoI	BstAPI
--------	------	--------

TAGTTGACATCCTTATAAATGGAATGTGCACATTTGCCAACACTGAGTGGCTTTCATCTGGAGCAGACTTTCGAGTCGTGGACTGCACACACATTTGCCTTTATG

100

TGTAACCTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCAGGGGCCAGGAAGACTACGGGAGGCTACACCAACGTCAAATCAGAGGGGCCTGTG

FIG. 10G (Cont'd)

FIG. 10G (Cont'd)

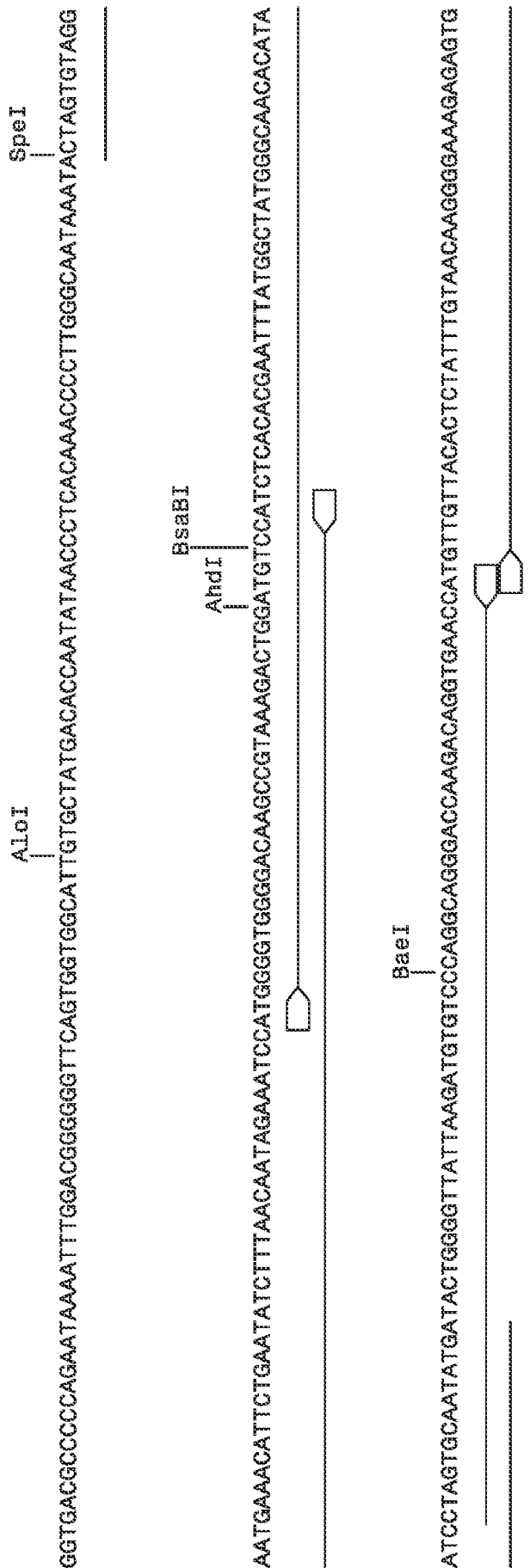


FIG. 10G (Cont'd)

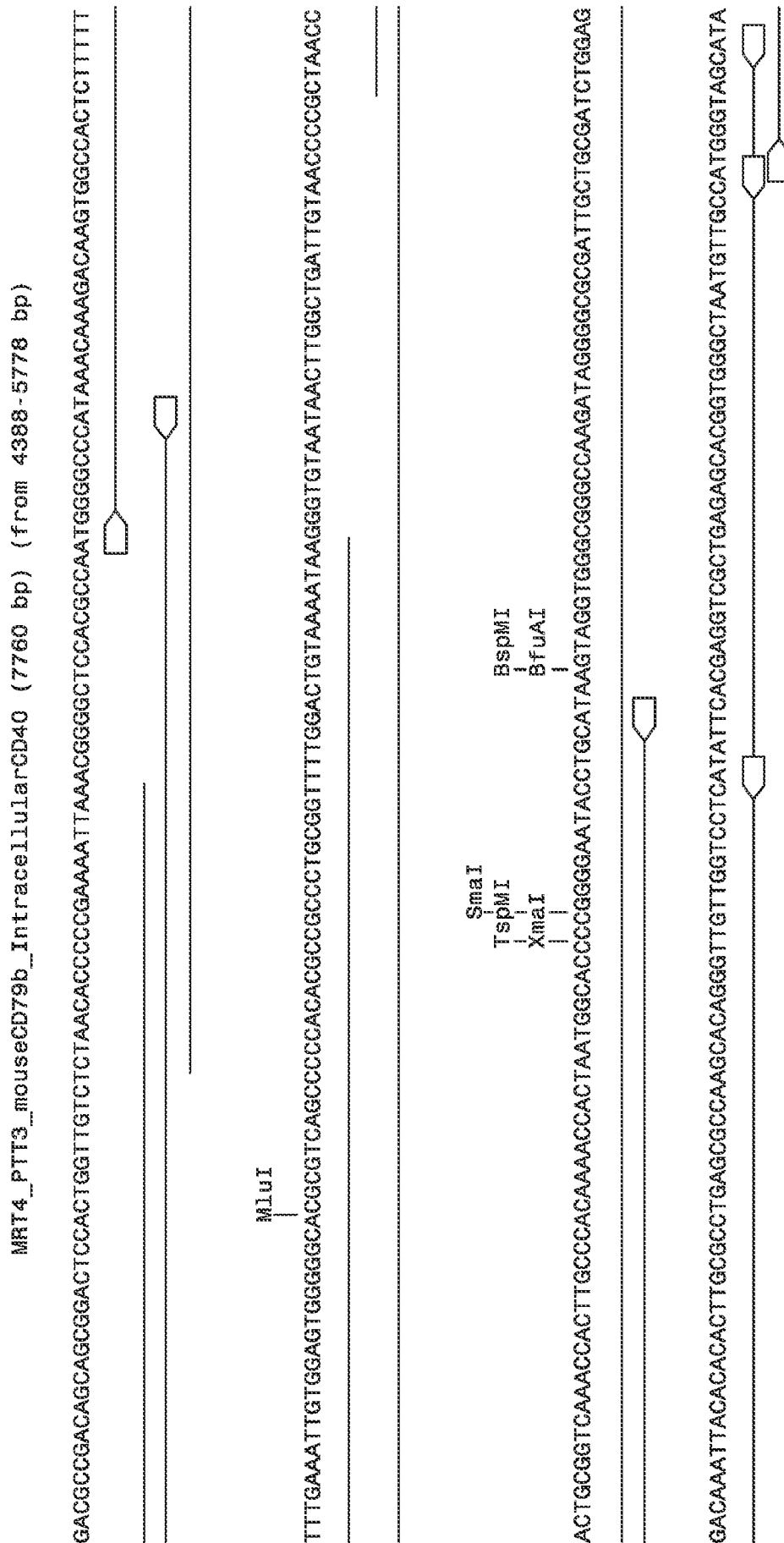


FIG. 10G (Cont'd)

TACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTG
GGTAGTATGCTATCCTAAATTTATCTGGGTAGCATAGGCTATCCTAATCTAATACTGGGTAGCATATGCTATGCTATGCTATCC
TAATCTGTATCCGGGTAGCATATGCTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATACTACCCAAATATCTGGATAGCAT
ATGCTATCCTAATCTATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTAT
ATCTGGGTAGTATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATGCT
TATCCTAATCTGTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTCTTGAAGACGAAAGGCCCTCGTGATACGCCCTATTTTATAGG
TTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTAT

FIG. 10G (Cont'd)

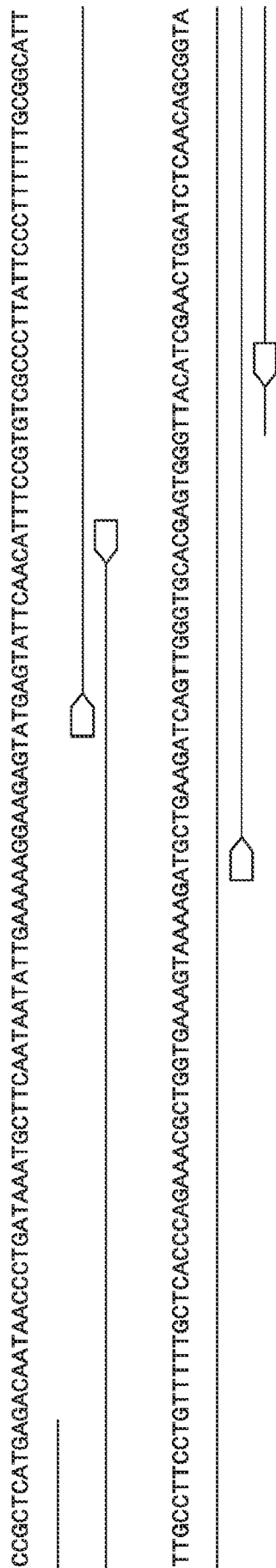


FIG. 10G (Cont'd)

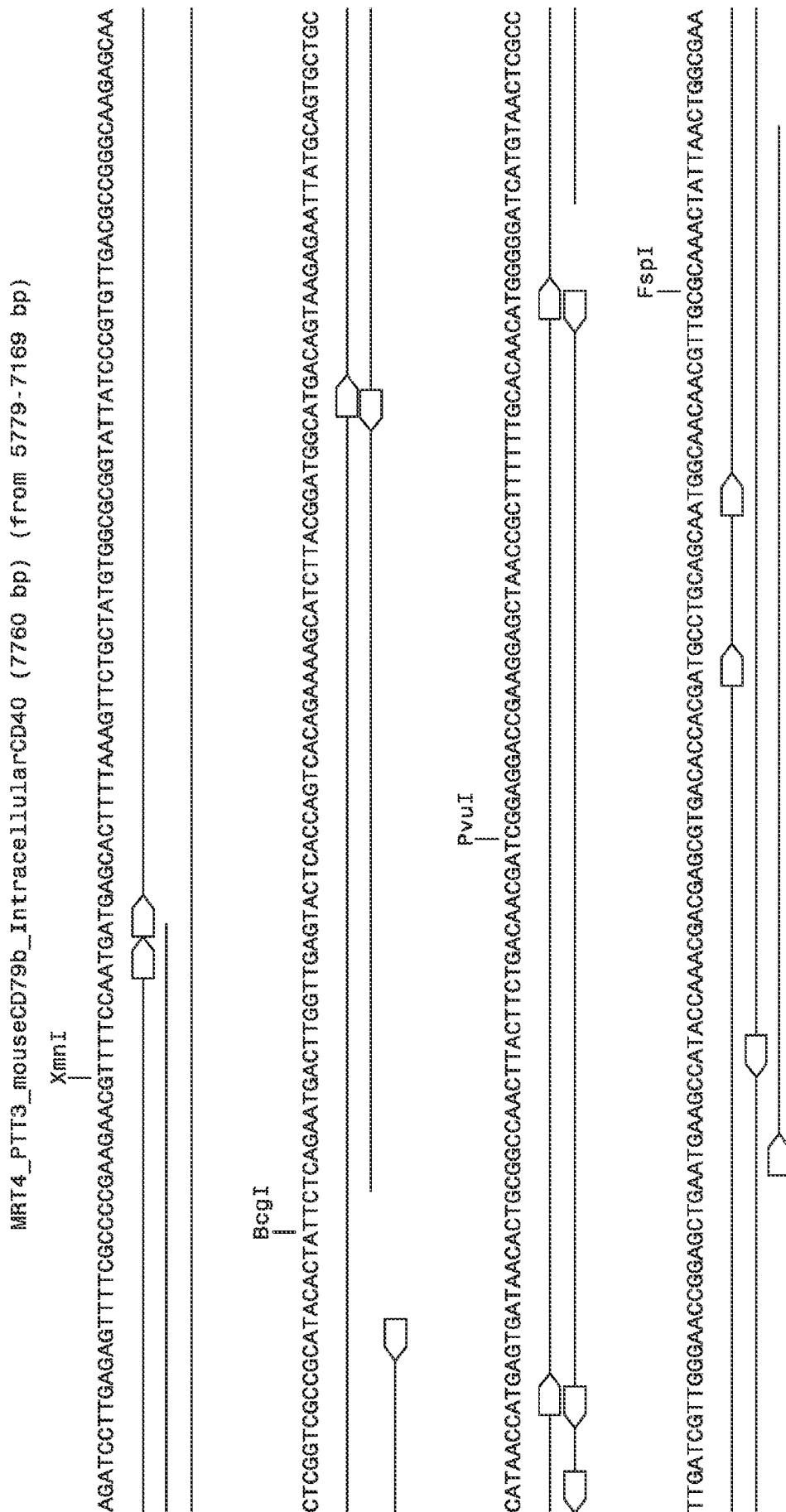


FIG. 10G (Cont'd)

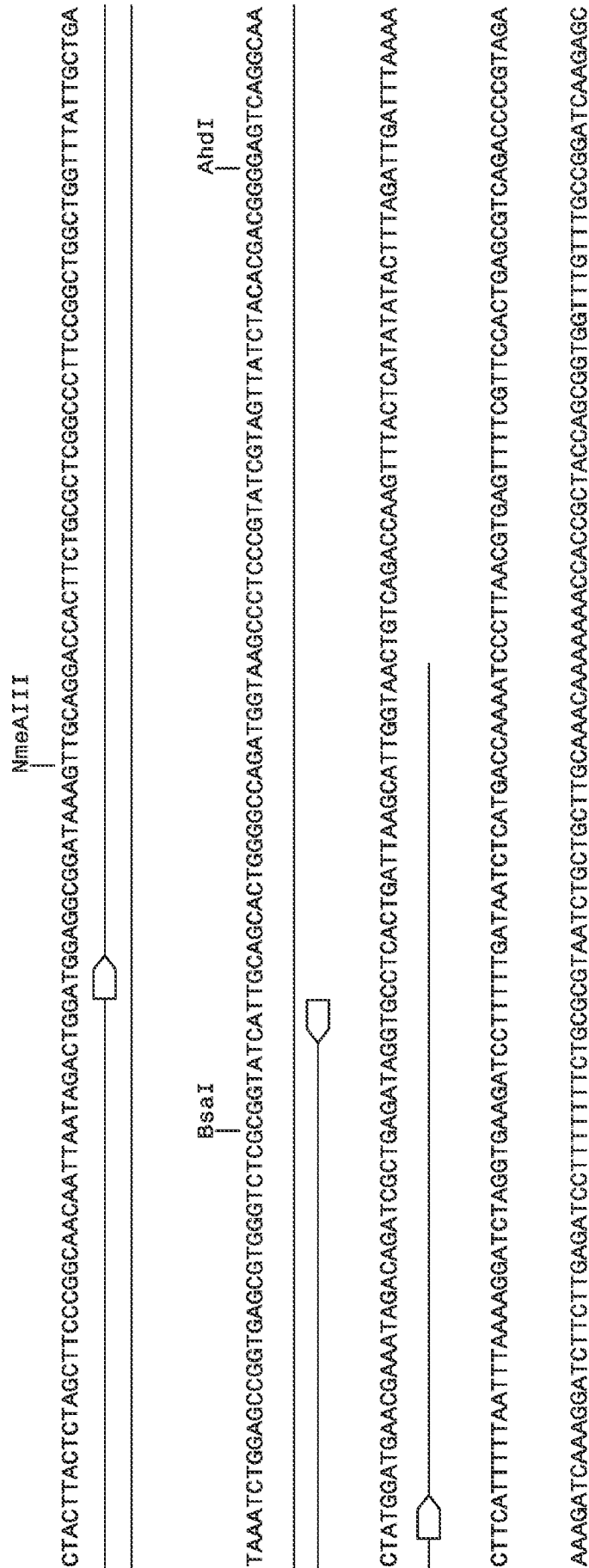


FIG. 10G (Cont'd)

TACCAACTCTTTTCCGGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACCTTCAAGAACTCTGTAGCA
CCGCCACATACCTCGGCTCGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTCCTTACCGGGTTGGACTCAAGAGACGATAGTTACCGGATAAGGC
GCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGGCCACGC
TTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGAGCTTCCAGGGGGAACCGCCTGGTATCTTTATAGTCCT



FIG. 10G (Cont'd)

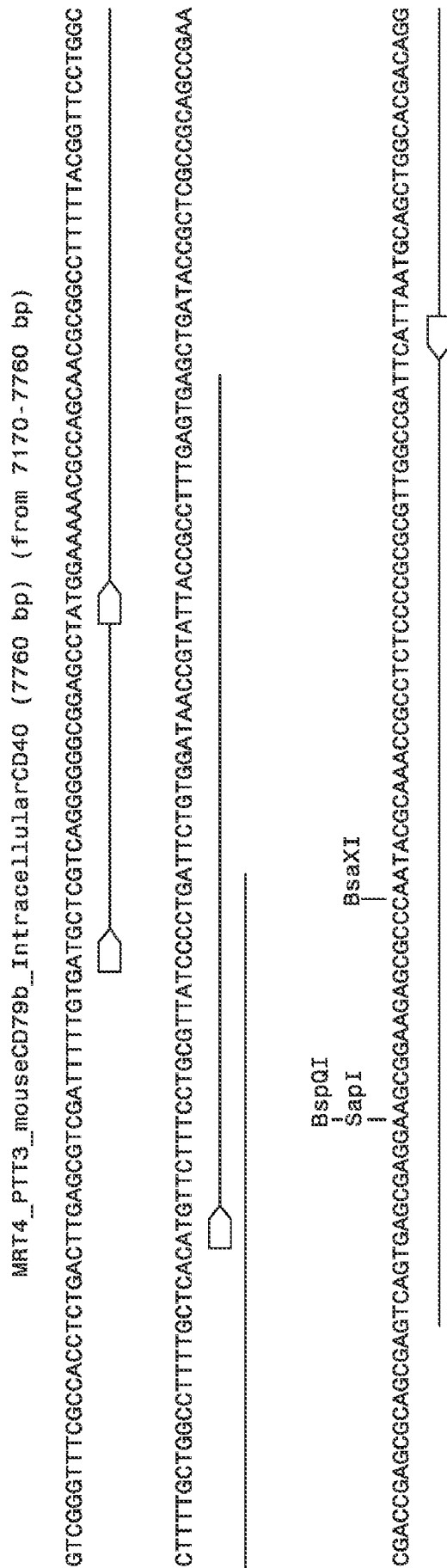
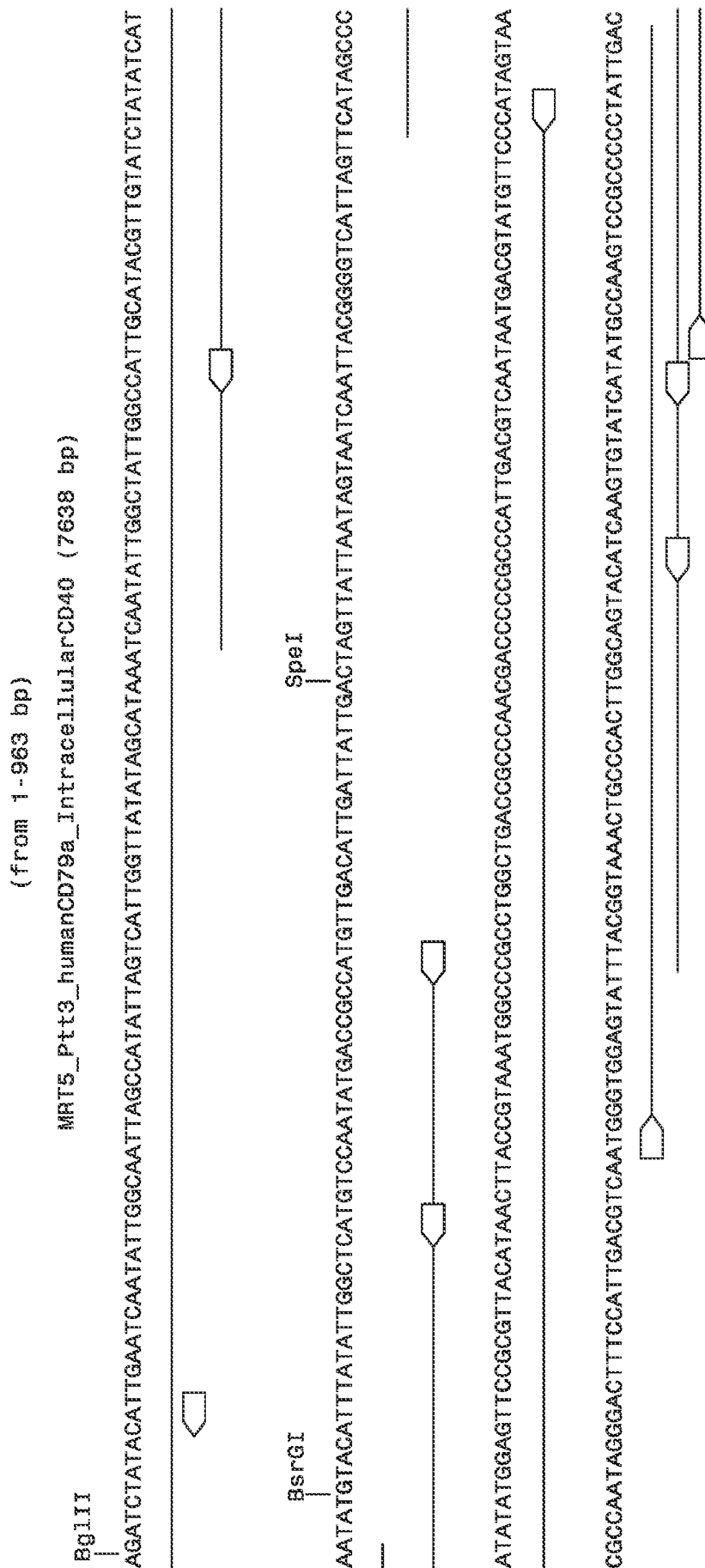


FIG. 10G (Cont'd)

(SEQ ID NO: 16)

FIG. 10G (Cont'd)



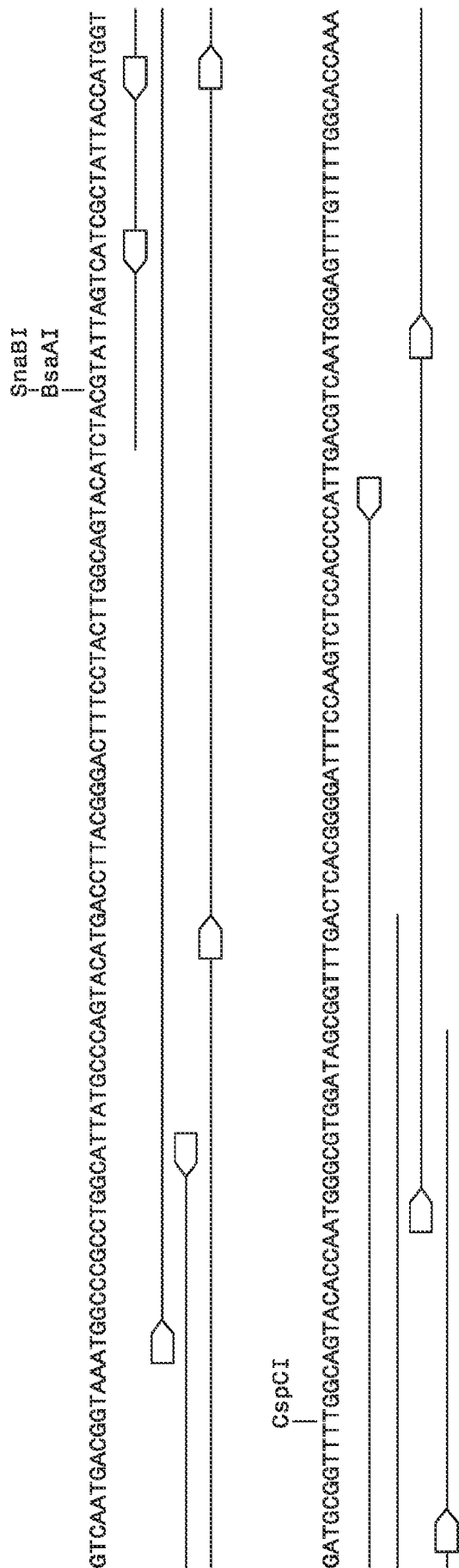


FIG. 10H (Cont'd)

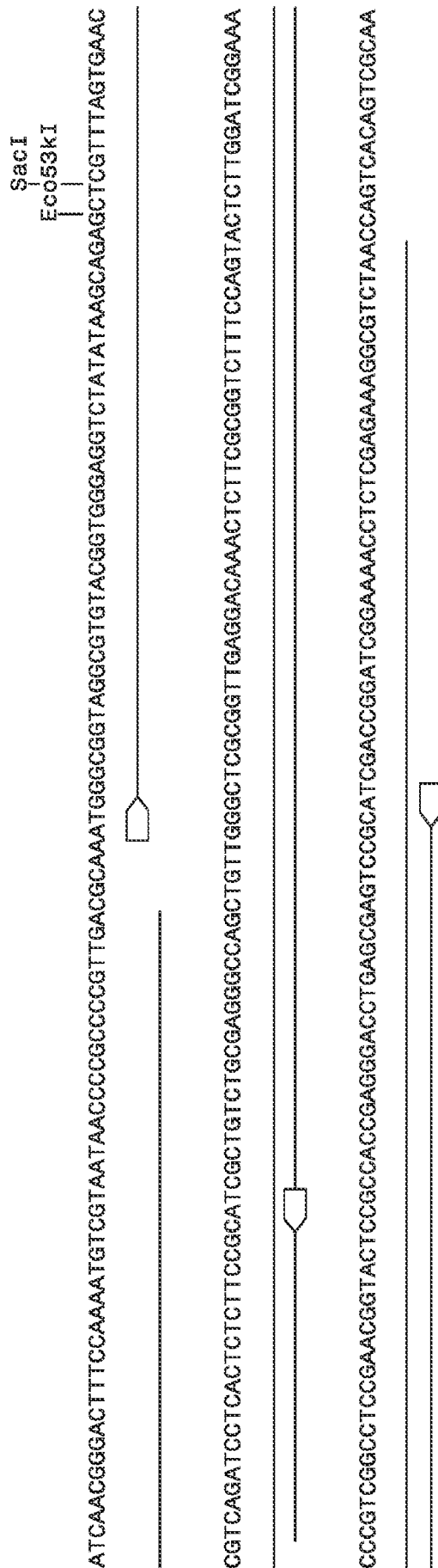


FIG. 10H (Cont'd)

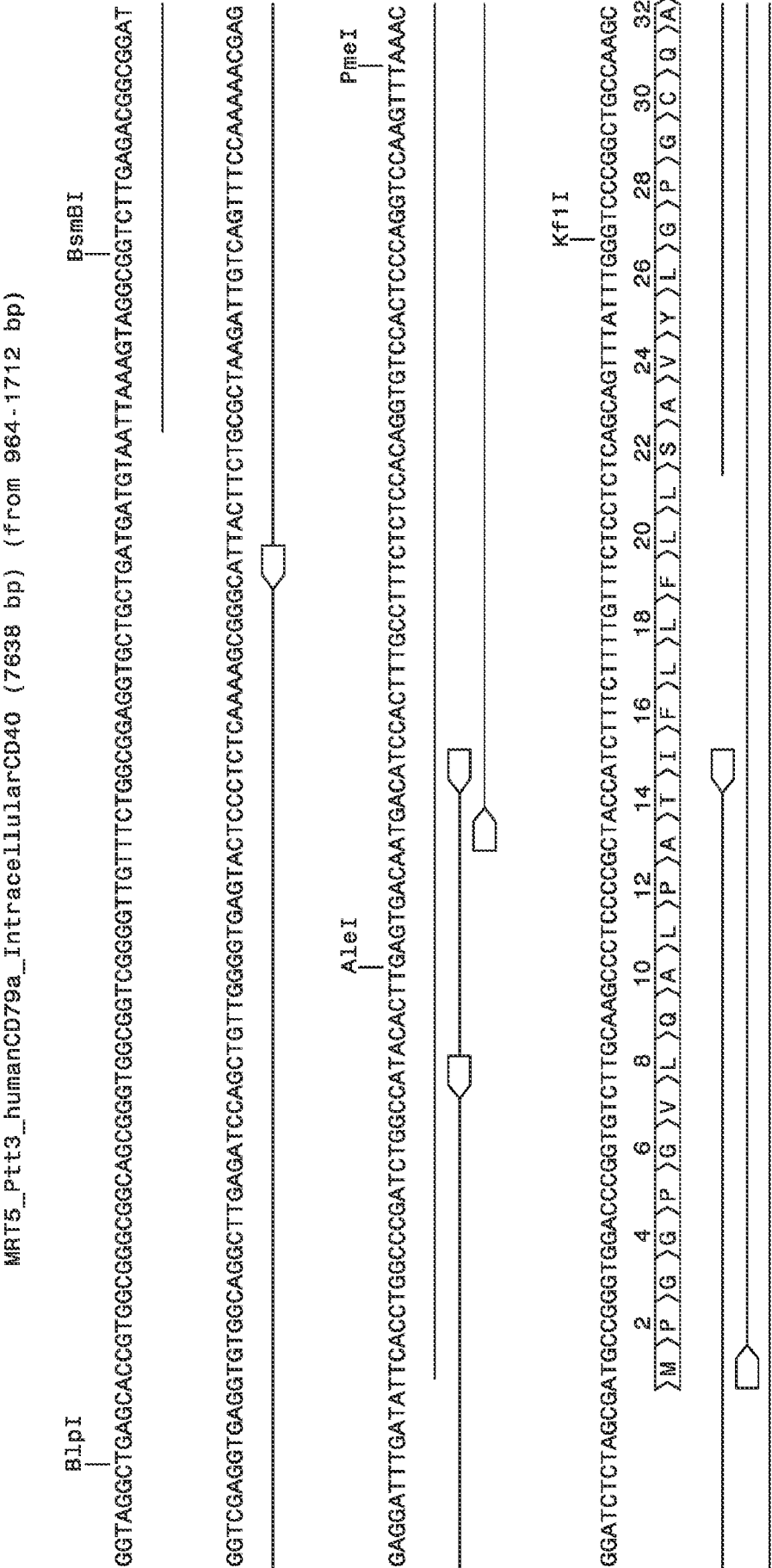


FIG. 10H (Cont'd)

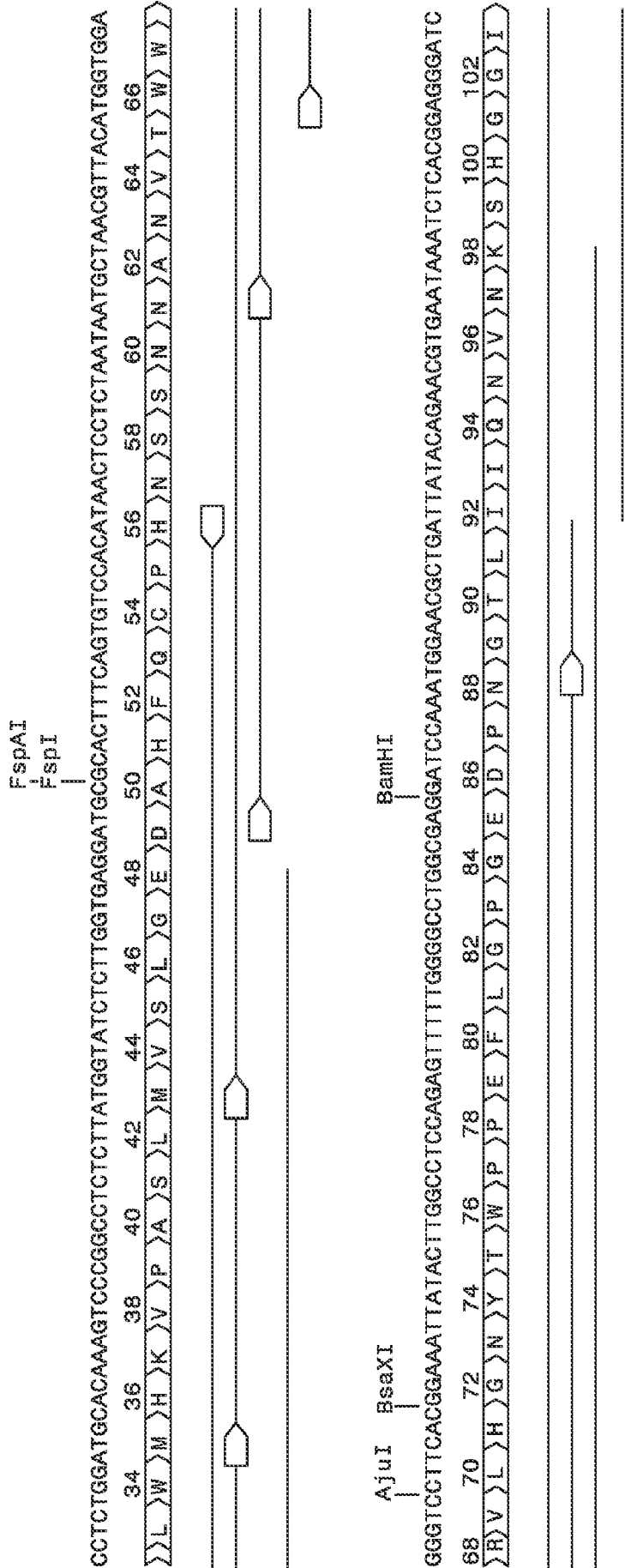


FIG. 10H (Cont'd)

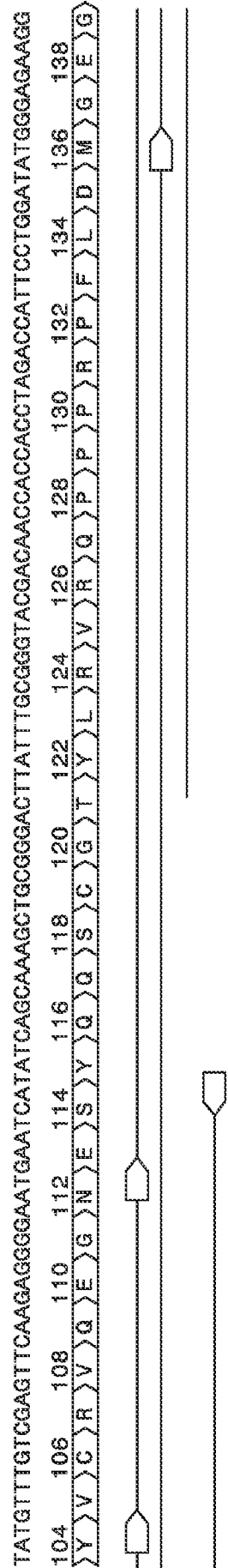


FIG. 10H (Cont'd)

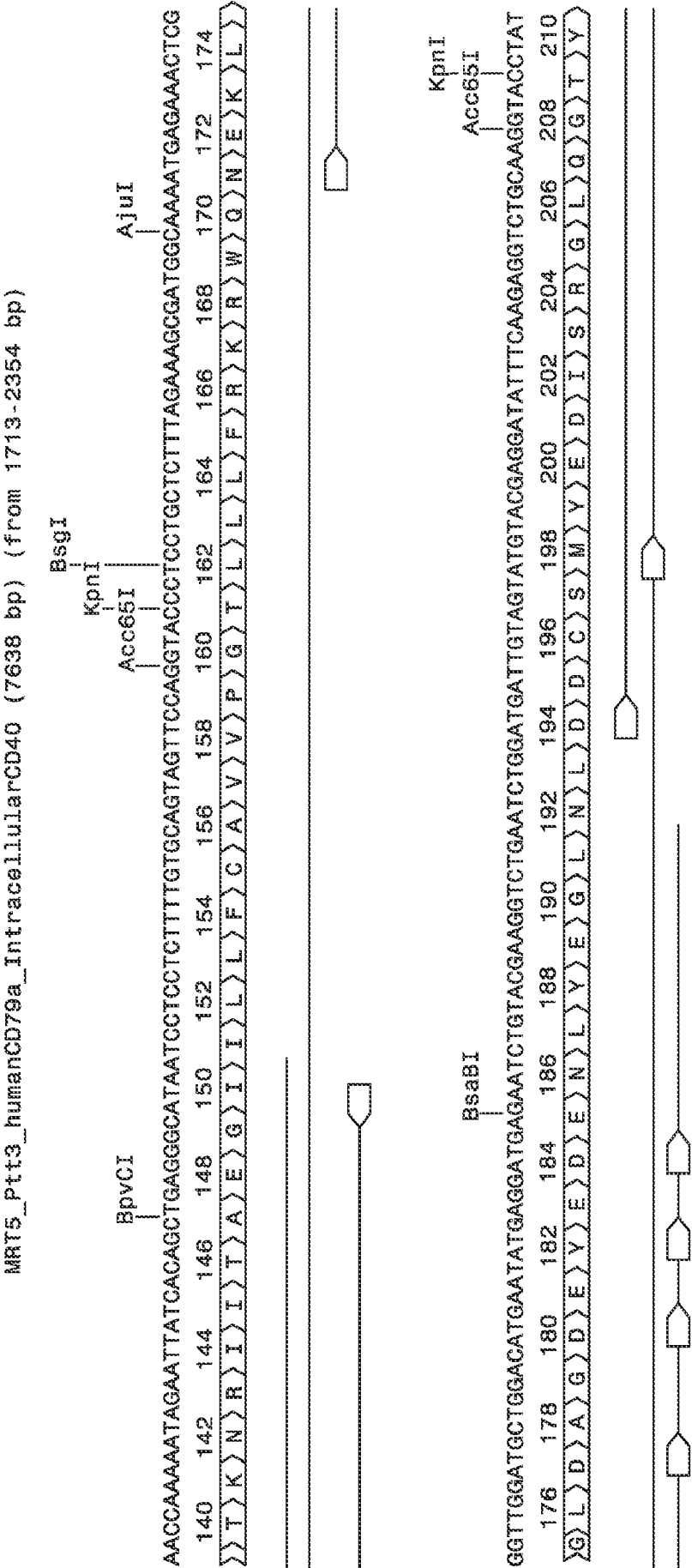


FIG. 10H (Cont'd)

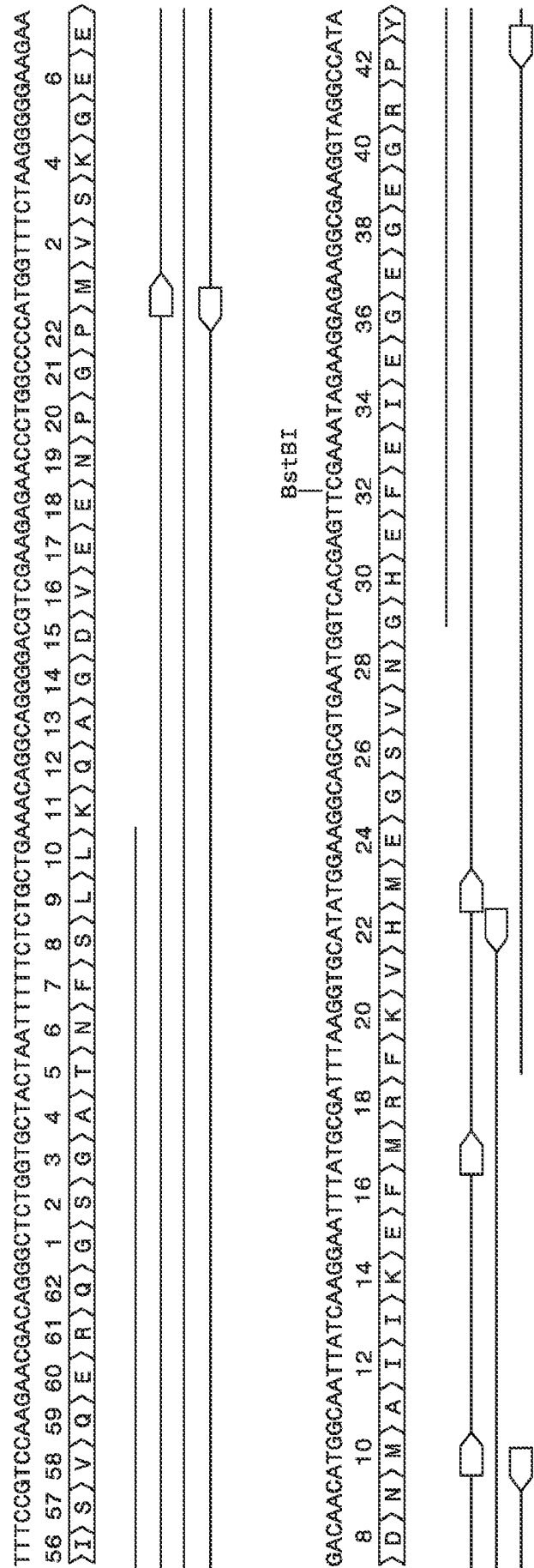


FIG. 10H (Cont'd)

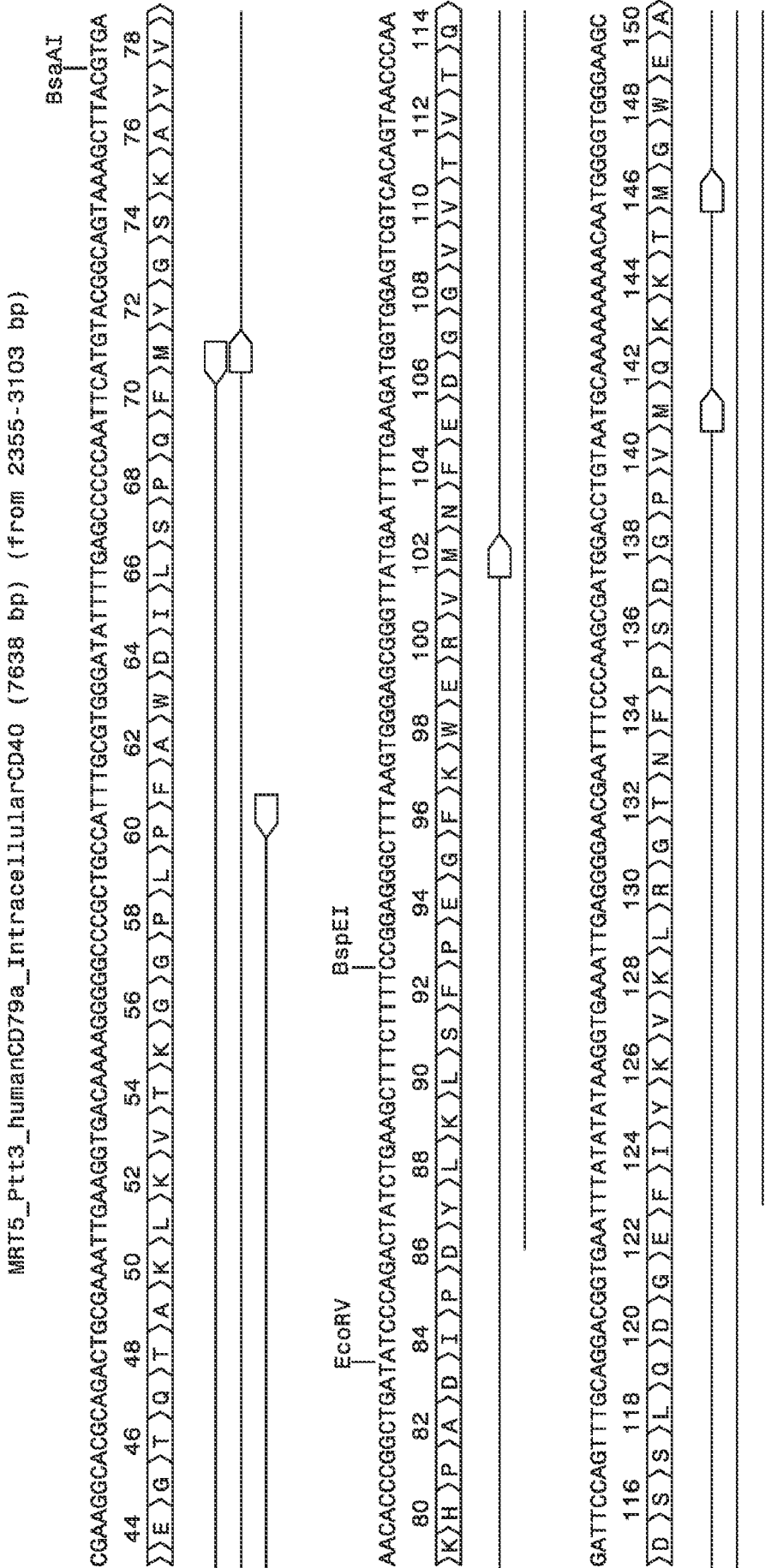


FIG. 10H (Cont'd)

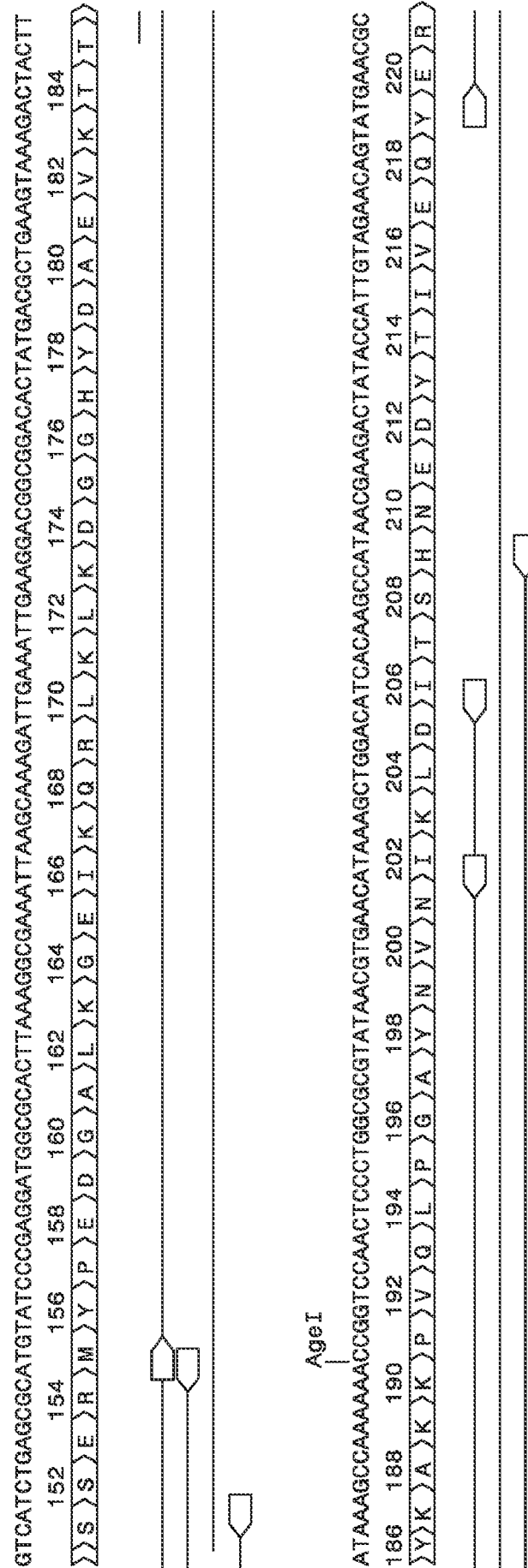


FIG. 10H (Cont'd)

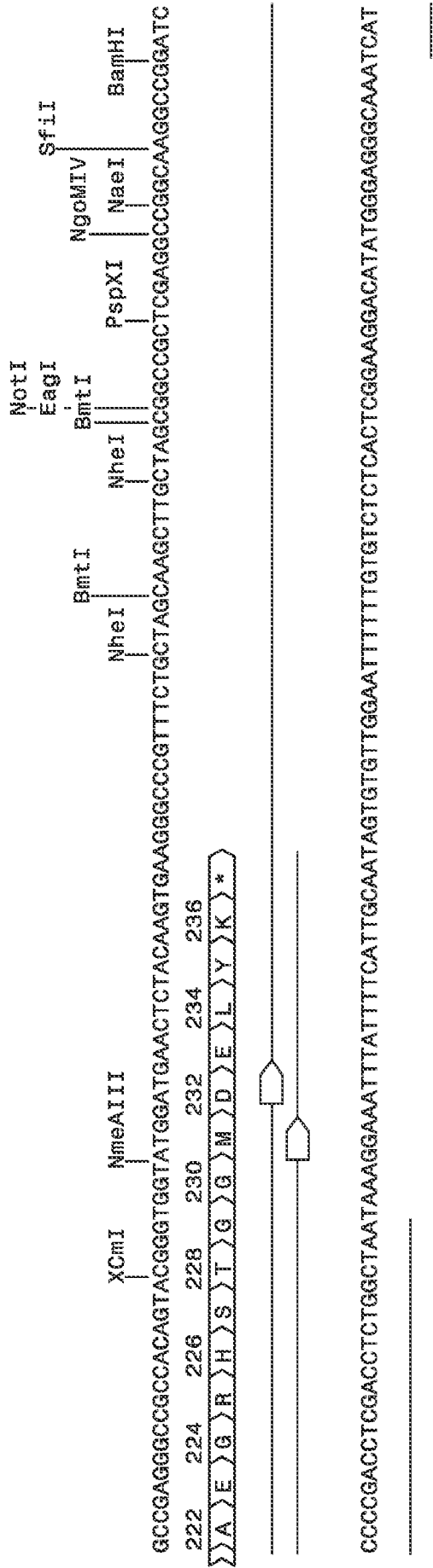


FIG. 10H (Cont'd)

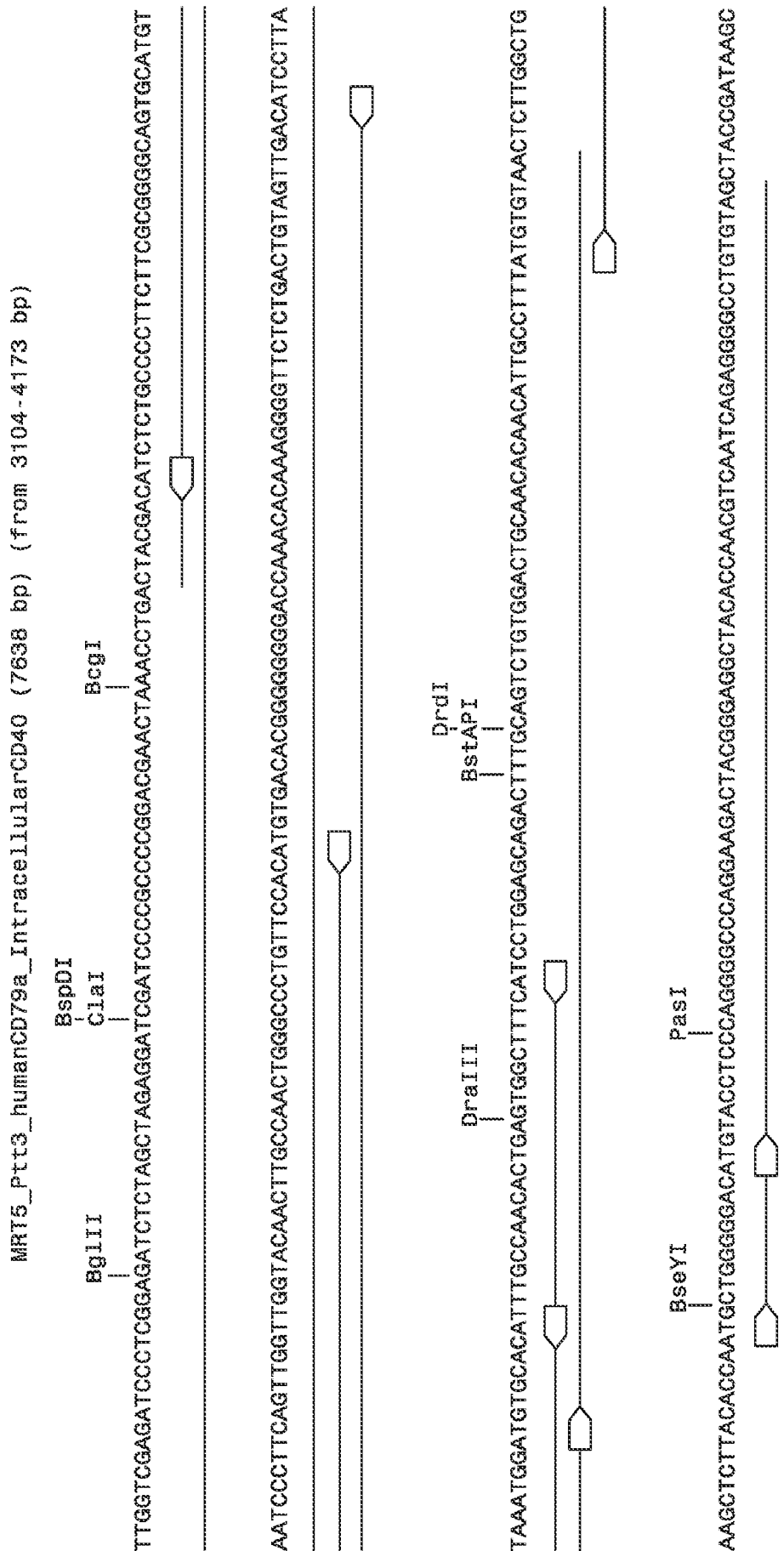


FIG. 10H (Cont'd)

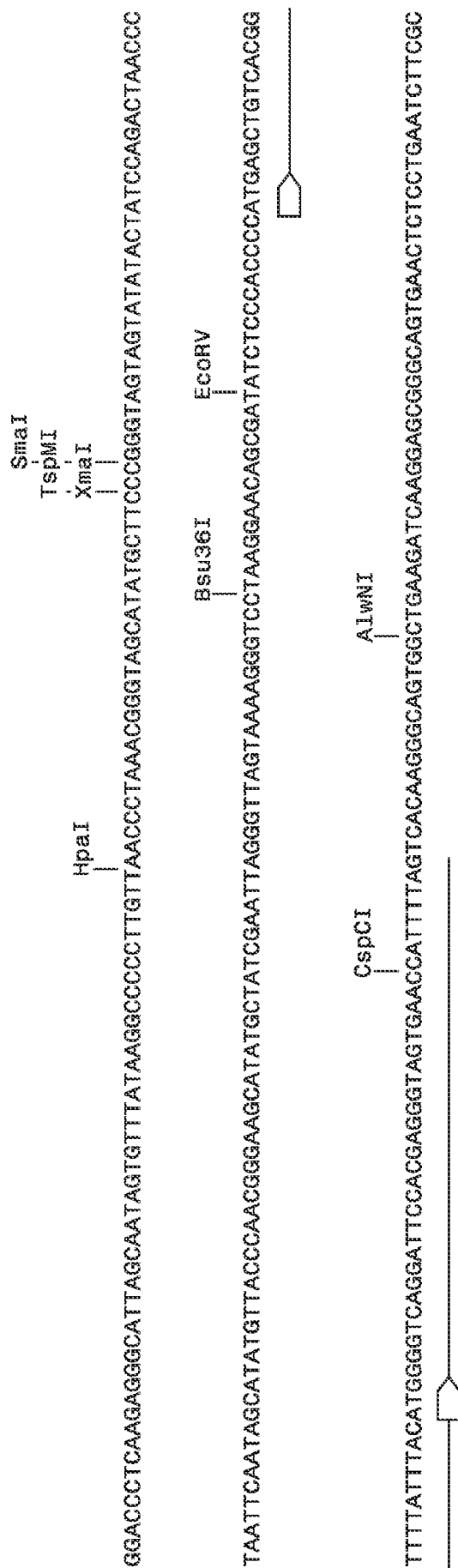


FIG. 10H (Cont'd)

FIG. 10H (Cont'd)

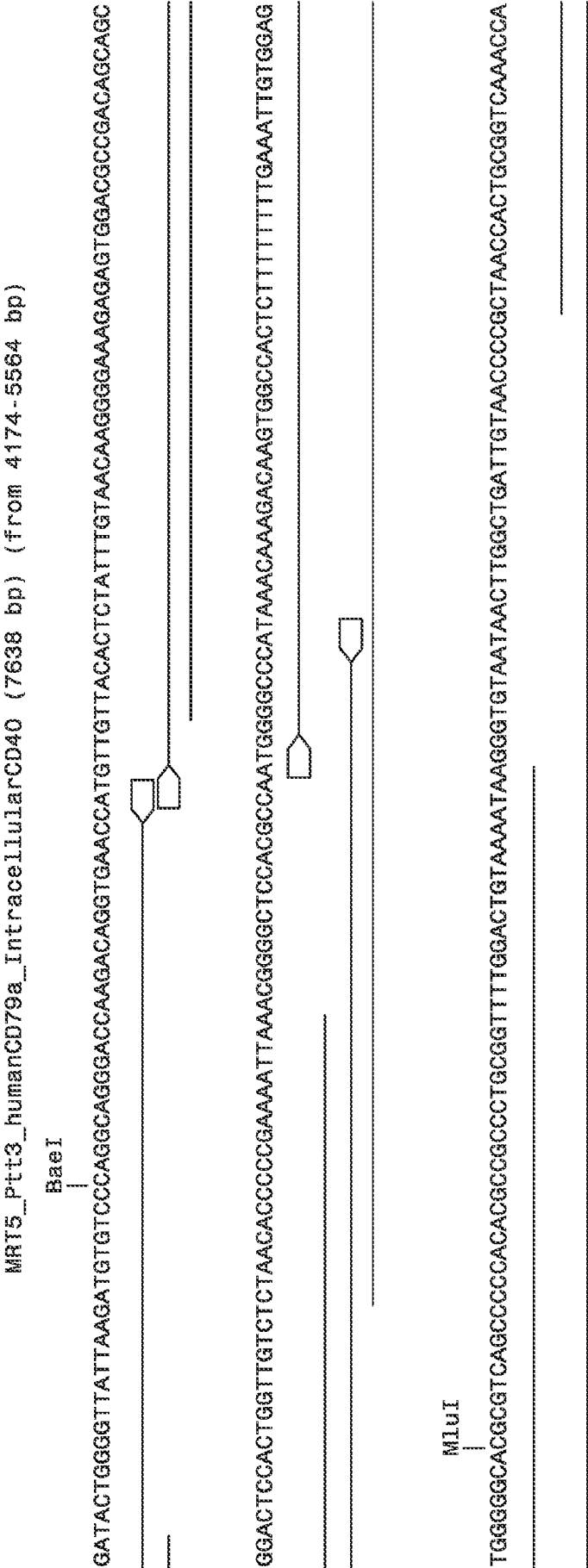


FIG. 10H (Cont'd)

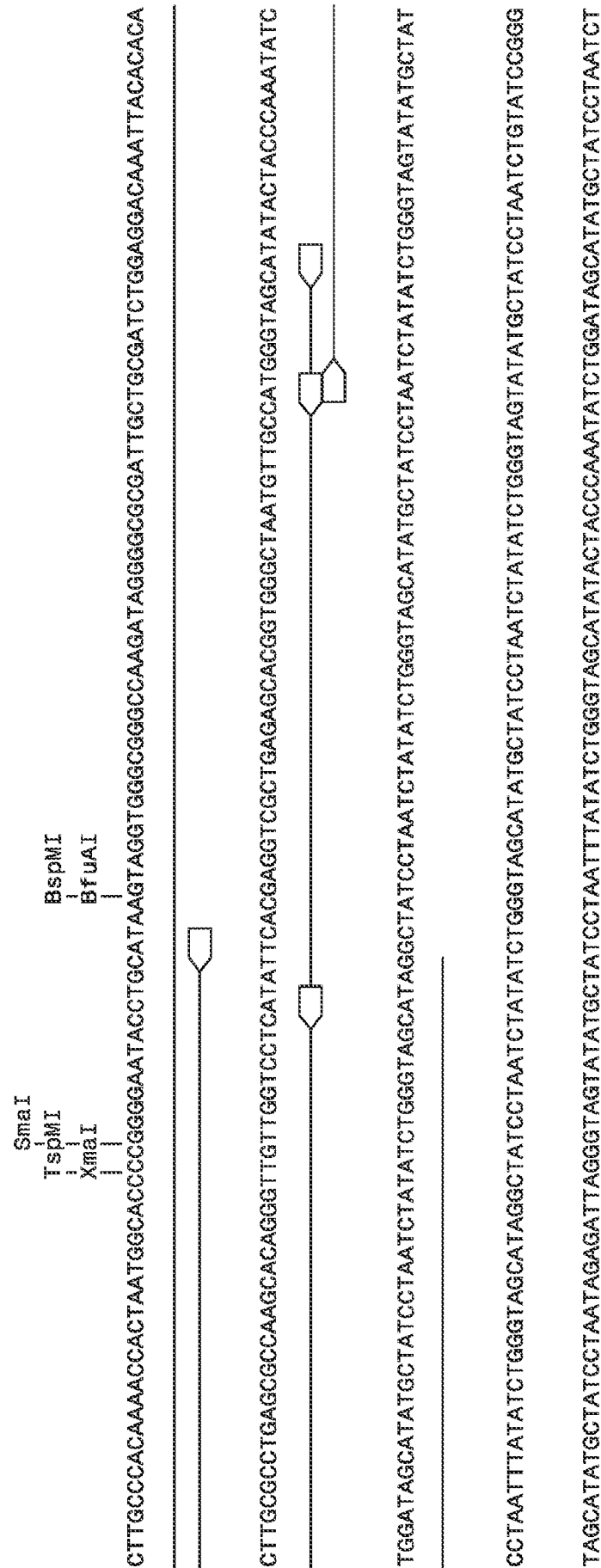


FIG. 10H (Cont'd)

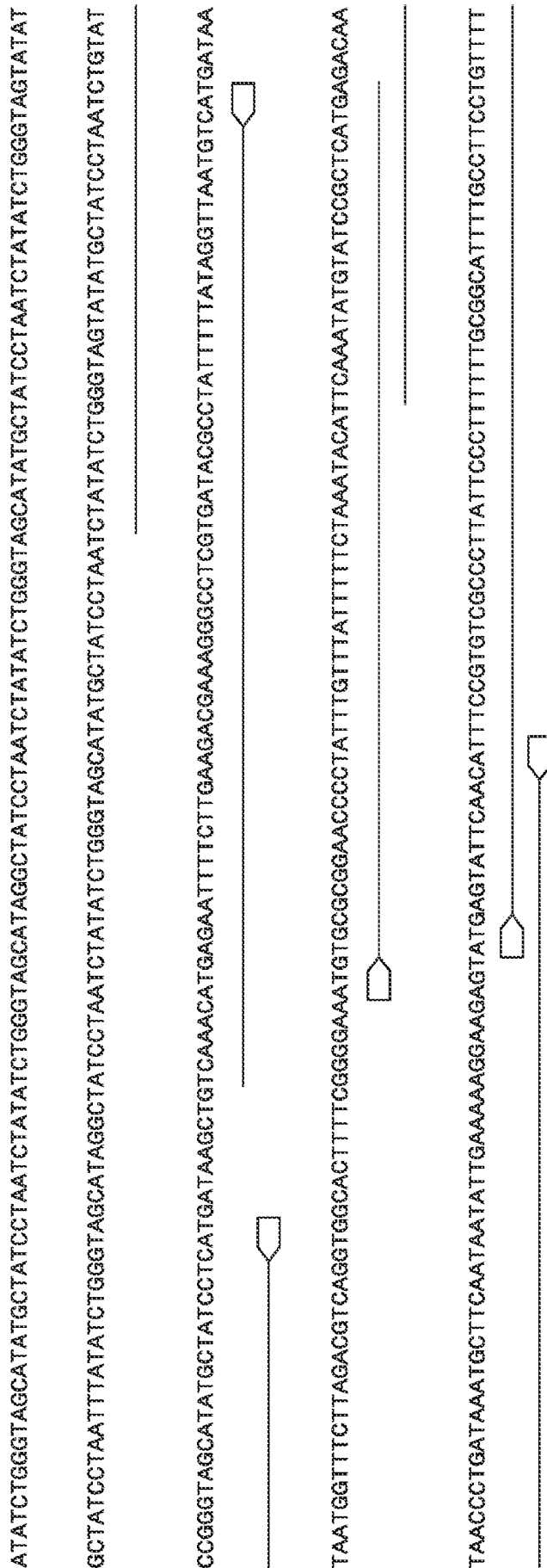


FIG. 10H (Cont'd)

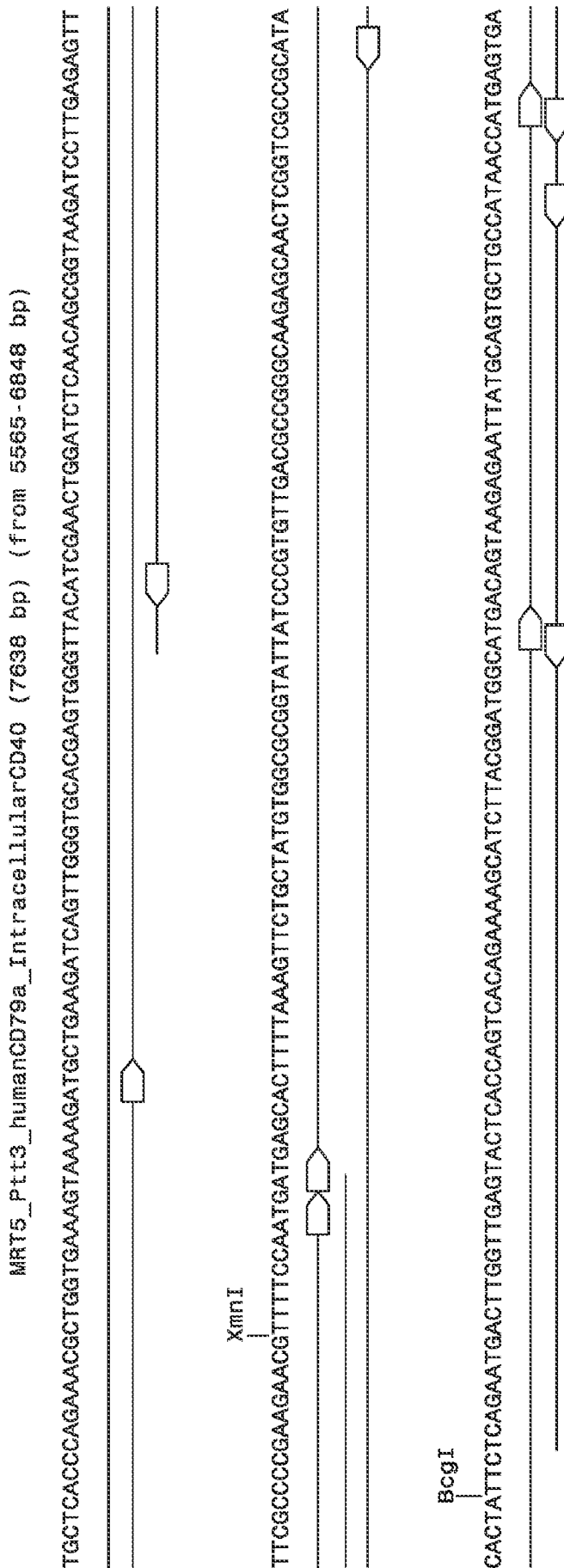


FIG. 10H (Cont'd)

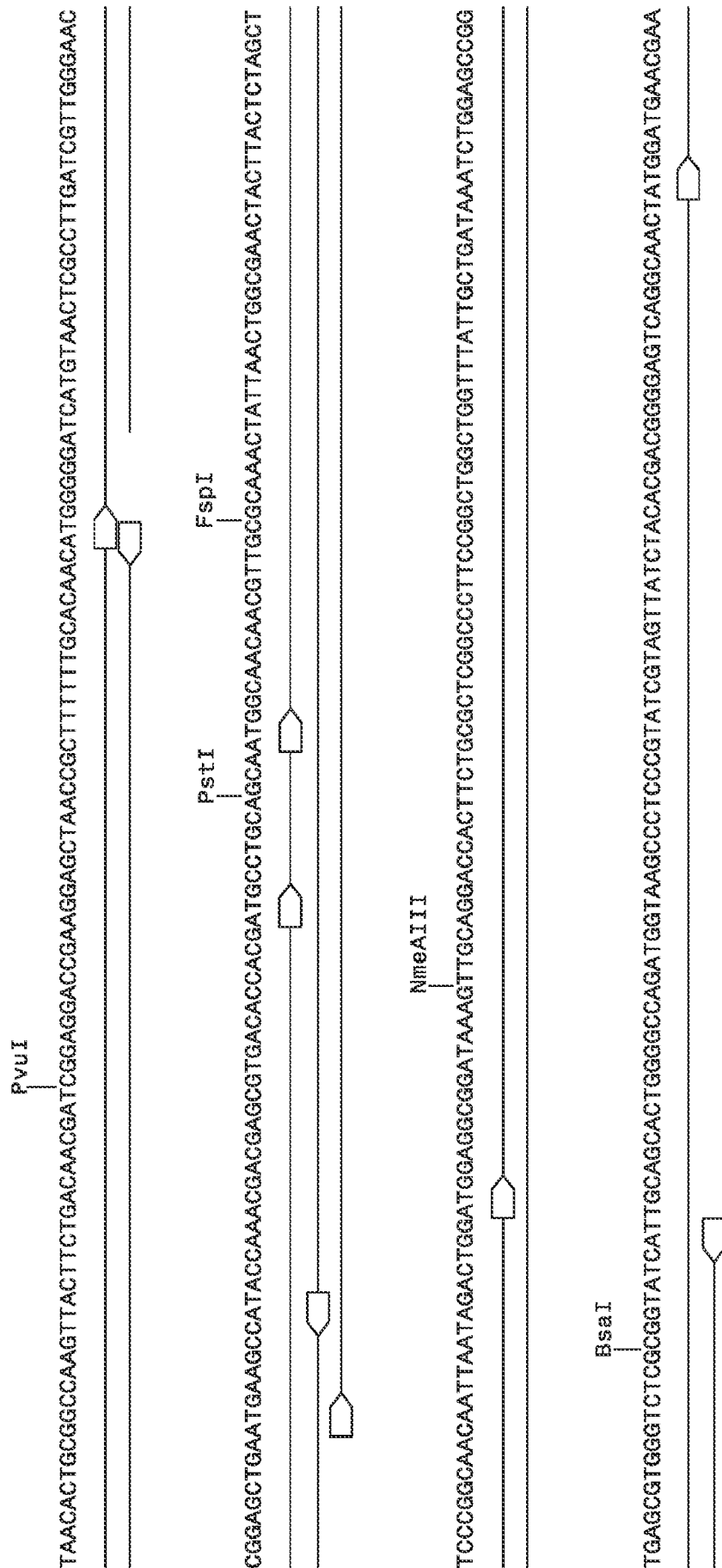


FIG. 10H (Cont'd)

ATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACCTTCATTTTTAATTT
AAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATC
TTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAAAAACCCGCTACCAGCGGTGGTTTGTGTTGCGGGAATCAAGAGCTACCAACTCTTTTTTC
CGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACGTCTCTTAGGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTC
GCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTCTTACCGGGTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGGCTG
ALWNI

FIG. 10H (Cont'd)

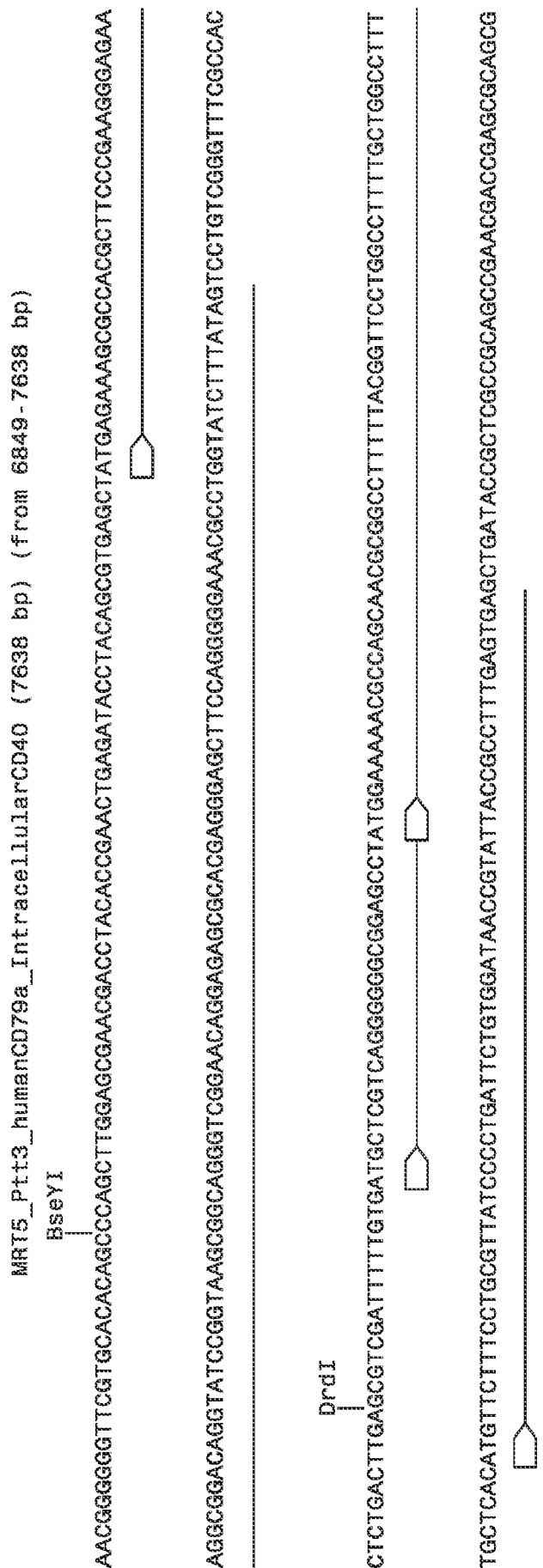


FIG. 10H (Cont'd)

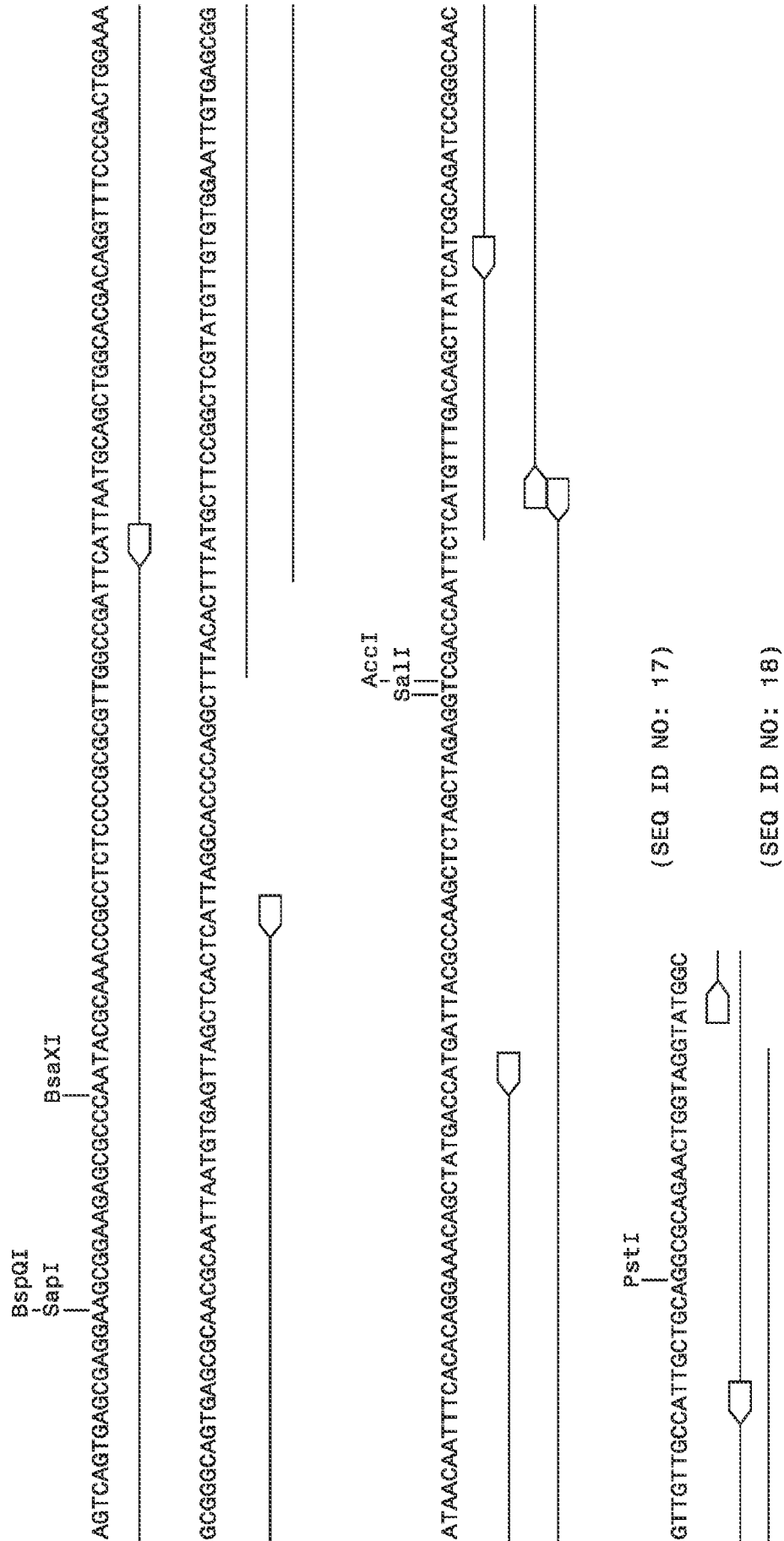


FIG. 10H (Cont'd)

Coding Sequence of Construct 1 - full CD40 (2520 bp)

CAAGTTTAAACGGATCTCTAGCGATGGTACGGCTCCCTCTGCAATGTGTACTTTGGGGATG
TCTTCTGACTGCCGTCCATCCCCAAGTACAGCTCGTACAAAGTGGAGCTGAGGTCAAAAAA
CCGGGTGCTTCAGTGAAAGTCTCATGTCAGGCTAGTGGGTATAGATTTAGTCATTTTACAG
TTCAGTGGGTTAGACAAGCACCTGGACAGAGGTTTGAATGGATGGGTTGGATAAACCATA
CAACGGTAATAAAGAGTTTCAGCGCGAAATTTCAAGACCGGGTAACTTTCACGGCTGACACA
AGCGCTAACACCGCGTACATGGAAGTGCAGAGTTTGGAGAGTGCTGACACCGCAGTATAC
TACTGTGCACGCGTTGGCGAGTGGGGATGGGATGACTCACCTACGACAATTATTATATG
GACGTTTGGGGTAAAGGCACCACTGTTATTGTCAGCTCAGGCGGTGGAGGGTCTGGAGGT
GGCGGTTCCGGTGGTGGAGGTAGCGAGATCGTTCTCACGCAAGCCCCAGGTACGCTGTC
ACTTTCTCCTGGTGAGCGAGCAACTTTTCAGTTGTAGATCCAGCCATTCCATTTCGCAGTAGA
AGAGTGGCCTGGTATCAACACAAGCCGGGCCAGGCACCTCGCCTTGTTATTCACGGGGTG
TCAAACCGCGCTTCAGGGATCTCAGATCGCTTCTCCGGCAGTGGGAGTGGAACCGACTTC
ACTTTGACTATCACTCGAGTTGAACCCGAGGATTTGCCTTGTTACTATTGCCAAGTTTACGG
GGCATCCTCTATACATTTGGACAGGGCACAAAGCTGGAGCGGAAAGGATCAGCGGGTAG
TGCAGCAGGGAGCGGCGAGTTCTGGTCTCATCCACAATTTGAAAAGGGAGGCGGAGGGG
GTGGTGAACCGCCAACAGCTTGCAGGGAGAAACAATACCTTATTAATAGTCAGTGCTGCTC
ACTGTGCCAGCCGGGTCAGAAGCTGGTCAGCGACTGTACGGAATTCAGTAAACGGAGTG
CCTGCCCTGCGGGGAAAGTGAATTCCTGGATACGTGGAATCGAGAACTCATTGTCACTAA
CACAAGTACTGTGACCCGAACCTGGGTCTGAGGGTGCAGCAAAAAGGCACCAAGTGAGACG
GATACAATTTGCACCTGTGAAGAGGGGCTGGCACTGTACCTCCGAGGCGTGCGAGAGCTGT
GTTCTCCACCGCTCTTGTTCTCCCGGATTTGGGGTTAAACAAATAGCAACTGGGGTTTCAG
ATACAATATGCGAACCATGCCCGGTCCGGTTTTTTTTCAAATGTCTCTAGCGCGTTTGAAAA
GTGTCATCCTTGGACAAGCTGCGAGACTAAGGACCTCGTCGTTTCAGCAAGCGGGTACGAA
TAAACTGATGTTGTTTGTGGGCCTCAGGATCGACTTAGAGCACTGGTTGTCATCCCTATTA
TATTTGGGATTTTGTTCGCTATCCTGCTTGCTTGTAAGAAGGTTGCTAAGAAGCCCACT
AACAAGGCGCCTCACCCGAAGCAGGAACCTCAAGAAATTAATTTTCCAGACGATCTGCCAG
GGTCAAACACGGCTGCACCCGTACAGGAAACACTGCACGGGTGTCAGCCCGTGACTCAAG
AAGACGGAAAAGAGAGCCGAATAAGCGTGCAGGAGCGACAAGGCGGTGGCGGCGGCGG
GTACCCCTACGACGTGCCAGATTATGCAGGCTCTGGTGCTACTAATTTTTCTCTGCTGAAA
CAGGCAGGGGACGTGCAAGAGAACCCTGGCCCCATGGTTTCTAAGGGGGAAGAAGACAA
CATGGCAATTATCAAGGAATTTATGCGATTTAAGGTGCATATGGAAGGCAGCGTGAATGGT
CACGAGTTCGAAATAGAAGGAGAAGGCGAAGGTAGGCCATACGAAGGCACGCAGACTGC
GAAATTGAAGGTGACAAAAGGGGGCCCGCTGCCATTTGCGTGGGATATTTTGAGCCCCCA
ATTCATGTACGGCAGTAAAGCTTACGTGAAACACCCGGCTGATATCCCAGACTATCTGAAG
CTTTCTTTTCCGGAGGGCTTTAAGTGGGAGCGGGTTATGAATTTTGAAGATGGTGGAGTCG
TCACAGTAACCCAAGATTCCAGTTTGCAGGACGGTGAATTTATATATAAGGTGAAATTGAG
GGGAACGAATTTCCCAAGCGATGGACCTGTAATGCAAAAAAACAATGGGGTGGGAAGC
GTCATCTGAGCGCATGTATCCCGAGGATGGCGCACTTAAAGGCGAAATTAAGCAAAGATTG
AAATTGAAGGACGGCGGACACTATGACGCTGAAGTAAAGACTACTTATAAAGCCAAAAAAC
CGGTCCAACCTCCCTGGCGCGTATAACGTGAACATAAAGCTGGACATCACAAGCCATAACG
AAGACTATACCATTGTAGAACAGTATGAACGCGCCGAGGGCCGCCACAGTACGGGTGGTA
TGGATGAACTCTACAAGTGAAGGGCCCGTTTCTGCTAGCAAG (SEQ ID NO: 3)

FIG. 11 cont'd

Construct 1 - full CD40 (humanCD40 signal peptide - anti-gp120 scFv - Linker - **StrepTagII** - Gly6 Linker- **full CD40 protein** - Gly6 Linker - *HA tag* - **P2A skipping element**- mCherry reporter)

MVRLPLQCVLWGCLLTAVHPQVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWRQAP
GQRFEWMGWINPYNGNKEFSAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEW
GWDDSPYDNYMDVWVGKGTIVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATF
SCRSSHSIRSRRAVWYQHKPGQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDF
ALYYCQVYGASSYTFGQGTKLERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPTACREKQ
YLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHKYCDPNLGLRVQ
KQGTSETDTICTCEEGWHCTSEACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVS
SAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGPQDRLRALVVIIFGILFAILLVLVKKVAKK
PTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGG
GYPYDVPDYAGSGATNFSLLKQAGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHMEGSVNG
HEFEIEGEGERPYEGTQAKLKVTKGGLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFP
EGFKWERVMNFEDGGVVTQDSSLQDGEFIKVKLRGTNFPSDGPVMQKKTMGWEASSER
MYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQ
YERAEGRHSTGGMDELYK^{*} (SEQ ID NO: 4)

Coding Sequence of Construct 2 - IgG ECD (2085 bp)

CAAGTTTAAACGGATCTCTAGCGATGGTACGGCTCCCTCTGCAATGTGTACTTTGGGGATG
TCTTCTGACTGCCGTCCATCCCCAAGTACAGCTCGTACAAAGTGGAGCTGAGGTCAAAAAA
CCGGGTGCTTCAGTGAAAGTCTCATGTCAGGCTAGTGGGTATAGATTTAGTCATTTTACAG
TTCAGTGGGTTAGACAAGCACCTGGACAGAGGTTTGAATGGATGGGTTGGATAAACCATA
CAACGGTAATAAAGAGTTCAGCGCGAAATTTCAAGACCGGGTAACTTTCACGGCTGACACA
AGCGCTAACACCGCGTACATGGAAGTTCGCAAGTTTGAAGAGTGCTGACACCGCAGTATAC
TACTGTGCACGCGTTGGCGAGTGGGGATGGGATGACTCACCTACGACAATTATTATATG
GACGTTTGGGGTAAAGGCACCACTGTTATTGTCAGCTCAGGCGGTGGAGGGTCTGGAGGT
GGCGGTTCCGGTGGTGGAGGTAGCGAGATCGTTCTCACGCAAGCCCCAGGTACGCTGTC
ACTTTCTCCTGGTGAGCGAGCAACTTTTCAAGTTGTAGATCCAGCCATTCCATTTCGAGTAGA
AGAGTGGCCTGGTATCAACACAAGCCGGGCCAGGCACCTCGCCTTGTTATTCACGGGGTG
TCAAACCGCGCTTCAGGGATCTCAGATCGCTTCTCCGGCAGTGGGAGTGGAAACCGACTTC
ACTTTGACTATCACTCGAGTTGAACCCGAGGATTTGCCTTGTTACTATTGCCAAGTTTACGG
GGCATCCTCTATACATTTGGACAGGGCACAAAGCTGGAGCGGAAAGGATCAGCGGGTAG
TGCAGCAGGGAGCGGCGAGTTCTGGTCTCATCCACAATTTGAAAAGGGAGGCGGAGGTG
GAGGTGAACCCAAGTCATGTGATAAGACACATACTTGTCCCCCTGCCCGGGAGGTGGTG
GAGGGGGTGCTCTCGTGGTTATCCCTATCATATTCGGCATACTTTTTGCAATCCTCCTTGTC
CTGGTAAAAAAGGTAGCGAAAAAGCCCAAGAAGGTTGCTAAGAAGCCCACTAACAAGGCG
CCTCACCCGAAGCAGGAACCTCAAGAAATTAATTTTCCAGACGATCTGCCAGGGTCAAACA
CGGCTGCACCCGTACAGGAAACACTGCACGGGTGTCAGCCCGTGAAGCAAGACGGA
AAAGAGAGCCGAATAAGCGTGCAGGAGCGACAAGGCGGTGGCGGGCGGGGTACCCCTA
CGACGTGCCAGATTATGCAGGCTCTGGTGCTACTAATTTTTCTCTGCTGAAACAGGCAGGG
GACGTCAAGAGAACCCTGGCCCCATGGTTTCTAAGGGGGAAGAAGACAACATGGCAATT
ATCAAGGAATTTATGCGATTTAAGGTGCATATGGAAGGCAGCGTGAATGGTCACGAGTTG
AAATAGAAGGAGAAGGCGAAGGTAGGCCATACGAAGGCACGCAGACTGCGAAATTGAAGG
TGACAAAAGGGGGCCCGCTGCCATTTGCGTGGGATATTTGAGCCCCCAATTCATGTACG

FIG. 11 cont'd

GCAGTAAAGCTTACGTGAAACACCCGGCTGATATCCCAGACTATCTGAAGCTTTCTTTTCC
 GGAGGGCTTTAAGTGGGAGCGGGTTATGAATTTTGAAGATGGTGGAGTCGTCACAGTAAC
 CCAAGATTCCAGTTTGCAGGACGGTGAATTTATATATAAGGTGAAATTGAGGGGAACGAAT
 TTCCCAAGCGATGGACCTGTAATGCAAAAAAACAATGGGGTGGGAAGCGTCATCTGAG
 CGCATGTATCCCGAGGATGGCGCACTTAAAGGCGAAATTAAGCAAAGATTGAAATTGAAGG
 ACGGCGGACACTATGACGCTGAAGTAAAGACTACTTATAAAGCCAAAAAACCGGTCCAAC
 CCCTGGCGCGTATAACGTGAACATAAAGCTGGACATCACAAGCCATAACGAAGACTATACC
 ATTGTAAGACAGTATGAACGCGCCGAGGGCCGCCACAGTACGGGTGGTATGGATGAACCT
 TACAAGTGAAGGGCCCGTTTCTGCTAGCAAG (SEQ ID NO: 5)

Construct 2 - IgG ECD (humanCD40 signal peptide - anti-gp120 scFv - Linker - **StrepTagII-
 Gly6 Linker- **IgG Hinge**- Gly6 Linker- CD40 Transmembrane Domain - **CD40 Intracellular**
 Domain - Gly6 Linker - HA tag - **P2A skipping element**- *mCherry reporter*)**

MVRLPLQCVLWGCLLTAVHPQVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWWRQAP
GQRFEEWMGWINPYNGNKEFSAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEW
GWDDSPYDNYMDVWVGKGTIVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATF
SCRSSHRSRRVAWYQHKPGQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDF
ALYYCQVYGASSYTFGQGTKLERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPKSCDKTHT
CPPCPGGGGGGALVVIPIIFGILFAILLVLVKKVAKKP**KKVAKKPTNKAPHPKQEPQEIFPDDL**
PGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGGGGYPYDVPDYAGSGATNFSLLK
QAGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKL
KVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQ
DSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGG
HYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK*
 (SEQ ID NO: 6)

Coding Sequence of Construct 3 - IgG ECD + TM (2082 bp)

CAAGTTTAAACGGATCTCTAGCGATGGTACGGCTCCCTCTGCAATGTGTACTTTGGGGATG
 TCTTCTGACTGCCGTCCATCCCCAAGTACAGCTCGTACAAAGTGGAGCTGAGGTCAAAAAA
 CCGGGTGCTTCAGTGAAAGTCTCATGTACAGGCTAGTGGGTATAGATTAGTCATTTTACAG
 TTTACTGGGTTAGACAAGCACCTGGACAGAGGTTTGAATGGATGGGTTGGATAAACCCATA
 CAACGGTAATAAAGAGTTTCAGCGCGAAATTTCAAGACCGGGTAACTTTCACGGCTGACACA
 AGCGCTAACACCGCGTACATGGAAGTTCGAAAGTTTGAGGAGTGCTGACACCGCAGTATAC
 TACTGTGCACGCGTTGGCGAGTGGGGATGGGATGACTCACCTACGACAATTATTATATG
 GACGTTTGGGGTAAAGGCACCACTGTTATTGTCAGCTCAGGCGGTGGAGGGTCTGGAGGT
 GGCGGTTCCGGTGGTGGAGGTAGCGAGATCGTTCTACGCAAGCCCCAGGTACGCTGTC
 ACTTTCTCCTGGTGAGCGAGCAACTTTTCAAGTTGTAGATCCAGCCATTCCATTCGCAGTAGA
 AGAGTGGCCTGGTATCAACACAAGCCGGGCCAGGCACCTCGCCTTGTTATTCACGGGGTG
 TCAAACCGCGCTTCAGGGATCTCAGATCGCTTCTCCGGCAGTGGGAGTGGAACCGACTTC
 ACTTTGACTATCACTCGAGTTGAACCCGAGGATTTGCGCTTGTTACTATTGCCAAGTTTACGG
 GGCATCCTCCTATACATTTGGACAGGGCACAAAGCTGGAGCGGAAAGGATCAGCGGGTAG
 TGCAGCAGGGAGCGGCGAGTTCTGGTCTCATCCACAATTTGAAAAGGGAGGGGGAGGCG
 GAGGAGAACCAAAAAGCTGTGACAAAACGCACACCTGCCCTCCCTGCCAGGTCTGTGGA
 CTACTATAACCATCTTTATCACGCTTTTTTTTGGCTTTTCAAGTTTGCTATAGCGCAACTATAACCT
 TTTTCGGGGGCGGCGGTGGTGGTAAGAAGGTTGCTAAGAAGCCCACTAACAAGGCGCCTC

FIG. 11 cont'd

ACCCGAAGCAGGAACCTCAAGAAATTAATTTTCCAGACGATCTGCCAGGGTCAAACACGGC
 TGCACCCGTACAGGAACACTGCACGGGTGTCAGCCCGTGA CTCAAGAAGACGGAAAAGA
 GAGCCGAATAAGCGTGCAGGAGCGACAAGGCGGTGGCGGCGGGGTACCCCTACGAC
 GTGCCAGATTATGCAGGCTCTGGTGTACTAATTTTCTCTGCTGAAACAGGCAGGGGACG
 TCGAAGAGAACCCTGGCCCCATGGTTTCTAAGGGGGAAGAAGACAACATGGCAATTATCA
 AGGAATTTATGCGATTTAAGGTGCATATGGAAGGCAGCGTGAATGGTCACGAGTTCGAAAT
 AGAAGGAGAAGGCGAAGGTAGGCCATACGAAGGCACGCAGACTGCGAAATTGAAGGTGA
 CAAAAGGGGGGCCCGCTGCCATTTGCGTGGGATATTTTGAGCCCCCAATTCATGTACGGCA
 GTAAAGCTTACGTGAAACACCCGGCTGATATCCCAGACTATCTGAAGCTTTCTTTTCCGGA
 GGGCTTTAAGTGGGAGCGGGTTATGAATTTTGAAGATGGTGGAGTCGTCACAGTAACCCA
 AGATTCCAGTTTGCAGGACGGTGAATTTATATATAAGGTGAAATTGAGGGGAACGAATTC
 CCAAGCGATGGACCTGTAATGCAAAAAAAAAACAATGGGGTGGGAAGCGTCATCTGAGCGC
 ATGTATCCCGAGGATGGCGCACTTAAAGGCGAAATTAAGCAAAGATTGAAATTGAAGGACG
 GCGGACACTATGACGCTGAAGTAAAGACTACTTATAAAGCCAAAAAACCGGTCCAACCTCC
 TGGCGCGTATAACGTGAACATAAAGCTGGACATCACAAGCCATAACGAAGACTATACCATT
 GTAGAACAGTATGAACGCGCCGAGGGCCGCCACAGTACGGGTGGTATGGATGAACTCTAC
 AAGTGAAGGGCCCGTTTCTGCTAGCAAG (SEQ ID NO: 7)

**Construct 3 - IgG ECD + TM (humanCD40 signal peptide - anti-gp120 scFv - Linker -
 StrepTagII- Gly6 Linker- *IgG Hinge*- Gly6 Linker- IgG2 Transmembrane Domain - Gly6 Linker -
 CD40 Intracellular Domain - Gly6 Linker - HA tag - *P2A skipping element*- *mCherry reporter*)**
 MVRLPLQCVLWGCLLTAVHPQVQLVQSGAEVKKPGASVKVSCQASGYRFSHTVHWVRQAP
 GQRFEWMGWINPYNGNKEFSAKFQDRVFTADTSANTAYMELRSLRSADTAVYYCARVGEW
 GWDDSPYDNYMDVWVGKGTIVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATF
 SCRSSHRSRRVAWYQHKPGQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDF
 ALYYCQVYGASSYTFGQGTKLERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPKSCDKTHT
 CPPCPGLWTTITIFITLFLSVCYSATITFFGGGGGGKKVAKKPTNKAPHPKQEPQEIFPDDL
 GSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGGGGYPYDVPDYAGSGATNFSLLKQ
 AGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLK
 VTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQ
 DSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGG
 HYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK*
 (SEQ ID NO: 8)

Coding Sequence of Construct 4 - full CD40+FKBP12v36 (3225 bp)

CAAGTTTAAACGGATCTCTAGCGATGGTACGGCTCCCTCTGCAATGTGTACTTTGGGGATG
 TCTTCTGACTGCCGTCCATCCCCAAGTACAGCTCGTACAAAGTGGAGCTGAGGTCAAAAAA
 CCGGGTGCTTCAGTGAAAGTCTCATGTACAGGCTAGTGGGTATAGATTAGTCATTTTACAG
 TTCACTGGGTTAGACAAGCACCTGGACAGAGGTTTGAATGGATGGGTTGGATAAACCATA
 CAACGGTAATAAAGAGTTCAGCGCGAAATTTCAAGACCGGGTAACCTTTCACGGCTGACACA
 AGCGCTAACACCGCGTACATGGAAGTTCGGAAGTTTGAGGAGTGCTGACACCGCAGTATAC
 TACTGTGCACGCGTTGGCGAGTGGGGATGGGATGACTCACCTACGACAATTATTATATG
 GACGTTTGGGGTAAAGGCACCACTGTTATTGTCAGCTCAGGCGGTGGAGGGTCTGGAGGT
 GGCGGTTCCGGTGGTGGAGGTAGCGAGATCGTTCTCACGCAAGCCCCAGGTACGCTGTC
 ACTTTCTCCTGGTGAGCGAGCAACTTTCAGTTGTAGATCCAGCCATTCCATTCGCAGTAGA

FIG. 11 cont'd

AGAGTGGCCTGGTATCAACACAAGCCGGGCCAGGCACCTCGCCTTGTTATTCACGGGGTG
TCAAACCGCGCTTCAGGGATCTCAGATCGCTTCTCCGGCAGTGGGAGTGGAACCGACTTC
ACTTTGACTATCACTCGAGTTGAACCCGAGGATTTGCCTTGACTATTGCCAAGTTTACGG
GGCATCCTCCTATACATTTGGACAGGGCACAAAGCTGGAGCGGAAAGGATCAGCGGGTAG
TGCAGCAGGGAGCGGCGAGTTCTGGTCTCATCCACAATTTGAAAAGGGAGGCGGAGGGG
GTGGTGAACCGCCAACAGCTTGCAGGGGAGAAACAATACCTTATTAATAGTCAGTGCTGCTC
ACTGTGCCAGCCGGGTCAGAAGCTGGTCAGCGACTGTACGGAATTCAGTGAACCGGAGTG
CCTGCCCTGCGGGGAAAGTGAATTCTTGGATACGTGGAATCGAGAACTCATTGTCATCAA
CACAAGTACTGTGACCCGAACCTGGGTCTGAGGGTGCAGCAAAAAGGCACCAGTGAGACG
GATACAATTTGCACCTGTGAAGAGGGGCTGGCACTGTACCTCCGAGGCGTGCGAGAGCTGT
GTTCTCCACCGCTCTTGTTCTCCCGGATTTGGGGTTAAACAAATAGCAACTGGGGTTTCAG
ATACAATATGCGAACCATGCCCCGTGGGTTTTTTTTCAAATGTCTCTAGCGCGTTTGAAAA
GTGTCATCCTTGGACAAGCTGCGAGACTAAGGACCTCGTCGTTTCAGCAAGCGGGTACGAA
TAAACTGATGTTGTTTGTGGGCCTCAGGATCGACTTAGAGCACTGGTTGTCATCCCTATTA
TATTTGGGATTTTGTTTCGCTATCCTGCTTGCTTGTAAGAAGGTTGCTAAGAAGCCCACT
AACAAGGCGCCTCACCCGAAGCAGGAACCTCAAGAAATTAATTTCCAGACGATCTGCCAG
GGTCAAACACGGCTGCACCCGTACAGGAAACACTGCACGGGTGTCAGCCCGTGAAGTCAAG
AAGACGGAAAAGAGAGCCGAATAAGCGTGACAGGAGCGACAAGGAGGTGGCGGAGGAGG
CGGGGTGCAAGTAGAAACCATATCTCCTGGTGACGGACGGACGTTTCCCAAGCGAGGACA
AACGTGCGTGGTACACTACACCGGGATGCTGGAAGACGGGAAAAAAGTTGATAGCAGCAG
GGACCGAAATAAGCCATTCAAATTTATGCTGGGAAAGCAGGAAGTGATCCGAGGCTGGGA
AGAAGGAGTTGCCAGATGTCAGTAGGCCAGCGGGCTAAACTGACAATCTCACCCGACTA
TGCATACGGGGCAACGGGACACCCGGGAATTATTCCTCCTCACGCTACGCTGGTCTTTGA
CGTTGAATTGTTGAACTGGAGGGCGGGGGCGGATCAGGTGGTGGTGGATCAGGCGGAG
GTGGAAGTGGGGTTCAAGTGGAACAATTTCTCCGGGAGATGGCAGAACCTTTCCCAAAC
GCGGCCAGACCTGCGTGGTACATTACACTGGCATGCTGGAGGATGGTAAAAAAGTAGACT
CAAGCCGAGACAGGAACAAGCCTTTTAAATTTATGTTGGGCAAACAAGAGGTTATTCGCGG
GTGGGAAGAAGGGGTCGCGCAGATGTCCGTCCGCCAACGAGCTAAACTCACGATAAGTC
CGGATTATGCATATGGGGCTACCGGACACCCAGGCATTATCCCACCCACGCGACGCTTG
TCTTTGATGTTGAACTTTTGAATTTGGAAGGCGGCGGGGAGGTGGCTACCCCTACGACG
TGCCAGATTATGCAGGCTCTGGTGCTACTAATTTTTCTCTGCTGAAACAGGCAGGGGACGT
CGAAGAGAACCCTGGCCCCATGGTTTCTAAGGGGGAAGAAGACAACATGGCAATTATCAA
GGAATTTATGCGATTTAAGGTGCATATGGAAGGCAGCGTGAATGGTCACGAGTTCGAAATA
GAAGGAGAAGGCGAAGGTAGGCCATACGAAGGCACGCAGACTGCGAAATTGAAGGTGAC
AAAAGGGGGCCCGCTGCCATTTGCGTGGGATATTTTGAAGCCCCCAATTCATGTACGGCAG
TAAAGCTTACGTGAAACACCCGGCTGATATCCCAGACTATCTGAAGCTTTCTTTTCCGGAG
GGCTTTAAGTGGGAGCGGGTTATGAATTTTGAAGATGGTGGAGTCGTACAGTAACCCAA
GATTCCAGTTTGCAGGACGGTGAATTTATATATAAGGTGAAATTGAGGGGAACGAATTTCC
CAAGCGATGGACCTGTAATGCAAAAAAAAACAATGGGGTGGGAAGCGTCATCTGAGCGCA
TGTATCCCAGAGGATGGCGCACTTAAAGGCGAAATTAAGCAAAGATTGAAATTGAAGGACGG
CGGACACTATGACGCTGAAGTAAAGACTACTTATAAAGCCAAAAAACCGGTCCAACCTCCCT
GGCGCGTATAACGTGAACATAAAGCTGGACATCACAAGCCATAACGAAGACTATACCATTG
TAGAACAGTATGAACGCGCCGAGGGCCGCCACAGTACGGGTGGTATGGATGAACTCTACA
AGTGAAGGGCCCCGTTTCTGCTAGCAAG (SEQ ID NO: 9)

FIG. 11 cont'd

Construct 4 - full CD40+FKBP12v36 (humanCD40 signal peptide - anti-gp120 scFv - Linker - **StrepTagII** - Gly6 Linker- **full CD40 protein** - Gly6 Linker- **FKBP12v36** - GlySer Linker - **FKBP12v36**- Gly6 Linker - **HA tag** - **P2A skipping element**- mCherry reporter)

MVRLPLQCVLWGCLLTAVHPQVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWRQAP
QQRFEWMGWINPYNGNKEFSAKFQDRVTFADTSANTAYMELRSLRSADTAVYYCARVGEW
GWDDSPYDNYMDVWVGKTTVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATF
SCRSSHISRRVAWYQHKPGQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPDF
ALYYCQVYGASSYTFGQGTKLERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPPTACREKQ
YLINSQCCSLCQPGQKLVSDCTEFTETECPCGESEFLDTWNRETHCHQHXYCDPNLGLRVQ
QKGTSETDTICTCEEGWHCTSEACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVS
SAFEKCHPWTSCETKDLVVQQAGTNKTDVVCQPQDRLRALVVIPIIFGILFAILLVLVKKVAKK
PTNKAPHPKQEPQEIINFDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGGG
GGVQVETISPGDGRTFPFRGQTCVVHYTGMLLEDGKKVDSSRDNRNPKFKFMLGKQEVIRGWE
EGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGSGGGGSGGGG
SGVQVETISPGDGRTFPFRGQTCVVHYTGMLLEDGKKVDSSRDNRNPKFKFMLGKQEVIRGWE
EGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGGGGYPYDVPDYA
GSGATNFSLLKQAGDVEENPGPMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEG
RPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMN
FEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPMQKKTMGWEASSERMYPEDGALKG
EIKQRLKLKDGGHYDAEVKTTYKAKKPVLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHST
GGMDELYK* (SEQ ID NO: 10)

Coding Sequence of Construct 5 - full CD40 no scFv (1692 bp)

CAAGTTTAAACGGATCTCTAGCGATGGTACGGCTCCCTCTGCAATGTGTACTTTGGGGATG
TCTTCTGACTGCCGTCCATCCCGAACCGCCAACAGCTTGCAGGGAGAAACAATACCTTATT
AATAGTCAGTGCTGCTCACTGTGCCAGCCGGGTGAGAAGCTGGTCAGCGACTGTACGGAA
TTCAGTCAAACGGAGTGCCTGCCCTGCGGGGAAAGTGAATTCTTGATACGTGGAATCGA
GAAACTCATTGTCATCAACACAAGTACTGTGACCCGAACCTGGGTCTGAGGGTGCAGCAAA
AAGGCACCAAGTGAGACGGATACAATTTGCACCTGTGAAGAGGGCTGGCACTGTACCTCCG
AGGCGTGCGAGAGCTGTGTTCTCCACCGCTCTTGTTCTCCCGGATTTGGGGTTAAACAAAT
AGCAACTGGGGTTTCAGATACAATATGCGAACCATGCCCGGTGCGGTTTTTTTCAAATGTC
TCTAGCGCGTTTGAAAAGTGTCATCCTTGACAAGCTGCGAGACTAAGGACCTCGTCGTTT
AGCAAGCGGGTACGAATAAACTGATGTTGTTTGTGGGCCTCAGGATCGACTTAGAGCACT
GGTTGTCATCCCTATTATATTTGGGATTTTGTTCGCTATCCTGCTTGCTTGTAAGAAGG
TTGCTAAGAAGCCCACTAACAAGGCGCCTCACCCGAAGCAGGAACCTCAAGAAATTAATTT
TCCAGACGATCTGCCAGGGTCAAACACGGCTGCACCCGTACAGGAACACTGCACGGGTG
TCAGCCCGTGACTCAAGAAGACGGAAAAGAGAGCCGAATAAGCGTGCAGGAGCGACAAG
GCGGTGGCGGCGGGGTACCCCTACGACGTGCCAGATTATGCAGGCTCTGGTGCTACT
AATTTTTCTCTGCTGAAACAGGCAGGGGACGTCGAAGAGAACCCTGGCCCCATGGTTTCTA
AGGGGGAAGAAGACAACATGGCAATTATCAAGGAATTTATGCGATTTAAGGTGCATATGGA
AGGCAGCGTGAATGGTCACGAGTTCGAAATAGAAGGAGAAGGCGAAGGTAGGCCATACGA
AGGCACGCAGACTGCGAAATTGAAGGTGACAAAAGGGGGCCCGCTGCCATTTGCGTGGG
ATATTTTGAGCCCCCAATTCATGTACGGCAGTAAAGCTTACGTGAAACACCCGGCTGATAT
CCCAGACTATCTGAAGCTTTCTTTTCCGGAGGGCTTTAAGTGGGAGCGGGTTATGAATTTT
GAAGATGGTGGAGTCGTCACAGTAACCCAAGATTCCAGTTTGCAGGACGGTGAATTTATAT

FIG. 11 cont'd

ATAAGGTGAAATTGAGGGGAACGAATTTCCCAAGCGATGGACCTGTAATGCAAAAAAAAAAC
 AATGGGGTGGGAAGCGTCATCTGAGCGCATGTATCCCGAGGATGGCGCACTTAAAGGCGA
 AATTAAGCAAAGATTGAAATTGAAGGACGGCGGACACTATGACGCTGAAGTAAAGACTACT
 TATAAAGCCAAAAAACCGGTCCAACCTCCCTGGCGCGTATAACGTGAACATAAAGCTGGACA
 TCACAAGCCATAACGAAGACTATACCATTGTAGAACAGTATGAACGCGCCGAGGGCCGCC
 ACAGTACGGGTGGTATGGATGAACTCTACAAGTGAAGGGCCCGTTTCTGCTAGCAAG
 (SEQ ID NO: 11)

Construct 5 - full CD40 no scFv (human CD40 signal peptide - full CD40 protein - Gly6

Linker - HA tag - P2A skipping element- mCherry Reporter

MVRLPLQCVLWGCLLTAVHPEPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECCLPC
GESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHR
SCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVV
CGPQDRLRALVVIPIIFGILFAILLVLVKKVAKKPTNKAPHPKQEPQEIINFDDLPGSNTAAPVQ
ETLHGCQPVTQEDGKESRISVQERQGGGGGGYPYDVDPDYAGSGATNFSLLKQAGDVEENP
GPMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPF
AWDILSPQFMYGSKAYVKHPADIPDYKLKSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFI
YKVKLRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLKDDGGHYDAEVKTTY
KAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK* (SEQ ID NO: 12)

Coding Sequence of MRT3_PTT3_mouseCD79a_IntracellularCD40 (7736 bp)

AGATCTATACATTGAATCAATATTGGCAATTAGCCATATTAGTCATTGGTTATATAGCATAAA
 TCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTG
 GCTCATGTCCAATATGACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAA
 TTACGGGGTTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAAT
 GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATGACGTCAATAATGACGTATGTT
 CCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
 CTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAA
 TGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCTTACT
 TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGTATGCGGTTTTGGCAGTACA
 CCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTTCGAAGTCTCCACCCCATGACG
 TCAATGGGAGTTTGTGTTTGGCACCAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCC
 GCCCCGTTGACGCAAATGGGCGGTAGGCGGTGACGGTGGGAGGTCTATATAAGCAGAGC
 TCGTTTAGTGAACCGTCAGATCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTG
 TTGGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACC
 CGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGG
 ATCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCAC
 CGTGGCGGGCGGCAGCGGGTGGCGGTGCGGGTTGTTTCTGGCGGAGGTGCTGCTGATG
 ATGTAATTAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGTGGCAGGCTT
 GAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAGCGGGCATTACTTCTGCGCTAAG
 ATTGTCAGTTTCCAAAACGAGGAGGATTTGATATTCACCTGGCCCGATCTGGCCATACAC
 TTGAGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTCCAAG
 TTAAACGGATCTCTAGCGATGCCTGGTGGCCTGGAAGCCCTGCGCGCCCTGCCCTTGTT
 GCTTTTTCTCTCTTACGCTTGCTTGGTCCAGGTGTCAAGCACTCAGGGTCAAGGAGGG
 CCCCCAGCCTCACAGTGAATCTCGGTGAGGAAGCCCGGTTGACCTGTGAGAACACGGT

FIG. 11 cont'd

AGAAACCCCAACATCACCTGGTGGTTTTCTTTGCAATCAAACATCACCTGGCCTCCTGTCC
CACTGGGCCCCGGCCAAGGAACAACCTGGACAACCTGTTCTTTCCCGAGGTGAATAAGAACC
ACCGAGGACTCTATTGGTGCCAAGTTATAGAGAACAACATCCTTAAGCGAAGCTGCGGGA
CTTATCTTAGGGTTTCGCAACCCTGTCCCCAGACCATTCTTGACATGGGGGAAGGGACCA
AGAATCGAATAATCACAGCAGAGGGTATTATTCTGTTGTTTTGCGCTGTGGTGCCAGGTAC
ACTTCTGCTTTTTTCGGAAGCGGTGGCAAAACGAGAAGTTCGGCGTGGATATGCCTGACGA
TTACGAGGATGAGAACCCTGTACGAGGGGCTTAATCTGGATGATTGTAGTATGTACGAAGAC
ATCAGCAGAGGCCTGCAAGGGACATACCAAGACGTAGGGAACCTGCACATCGGTGATGCT
CAACTGGAGAAACCAAGAAAGTGGTCAAGAAGCCAAAGGACAATGAGATTCTGCCCCC
GCTGCAAGAAGGCAAGATCCCCAGGAGATGGAGGACTATCCAGGCCACAACACAGCCGC
CCCAGTGCAAGGAACTCTGCACGGTTGCCAACCTGTTACACAGGAGGATGGGAAGGAAAG
CCGCATCAGTGTGCAAGAGCGACAAGTGACAGACTCAATAGCCTTGCGGCCCTCGTTGG
CTCCGGCGCTACCAATTTTAGCCTGTTGAAGCAGGCTGGGGACGTGGAAGAGAACCCCGG
ACCAGGAAGTGGCGAGGGAAGGGGGAGTCTTCTTACCTGTGGGGATGTTGAAGAGAATCC
TGGCCCCATGGTATCCAAGGGAGAGGAGCTGTTACAGGCGTTGTCCCAATTCTCGTGGA
ATTGGATGGGGACGTAAATGGTCACAAGTTTAGCGTCTCTGGCGAGGGCGAGGGTGACGC
AACATATGGTAAATTGACTCTGAAGTTTATTTGCACCACAGGTAACTTCCTGTGCCTTGGC
CTACACTTGTCACTACTCTGACCTATGGCGTGCAATGTTTCTCCCGCTACCCTGACCATAT
GAAACAACACGATTTTTTTAAATCTGCCATGCCCGAAGGGTATGTGCAGGAACGGACTATT
TTCTTTAAGGACGACGGCAATTATAAACTCGAGCTGAGGTCAAATTTGAAGGTGACACCT
TGGTCAACAGAATAGAACTCAAGGGCATTGATTTTAAAGAGGACGGGAACATACTGGGTCA
CAAATTGGAGTACAATTATAACTCCCACAACGTTTATATAATGGCTGATAAGCAGAAAAATG
GAATTAAGGTCAACTTCAAATCCGACATAACATAGAGGATGGGTCAGTACAGCTTGCCGA
CCATTATCAGCAAAACACTCCTATAGGCGATGGACCCGTGCTTTTGCTGACAATCATTATC
TGTCTACTCAAAGCGCTCTTTCTAAAGATCCTAACGAAAAACGGGATCATATGGTGCTCTTG
GAGTTTGTAACTGCCGCCGGCATCACATTGGGAATGGACGAACTCTATAAATGACCCTCTA
GAGGGCCCGTTTCTGCTAGCAAGCTTGCTAGCGGCCGCTCGAGGCCGGCAAGGCCGGAT
CCCCGACCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA
TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTGGTTCGAGATCCCT
CGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCGGACGAACTAAACCTGACTACGACA
TCTCTGCCCCTTCTTCGCGGGGCAGTGCATGTAATCCCTTCAGTTGGTTGGTACAACCTTGC
CAACTGGGCCCTGTTCCACATGTGACACGGGGGGGACCAAAACACAAAGGGGTTCTCTGA
CTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTTCATCCT
GGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTTTATGTGTAACCTTTGGC
TGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCCAGGGGGCCAGGAAGACTACGG
GAGGCTACACCAACGTCAATCAGAGGGGCCTGTGTAGCTACCGATAAGCGGACCCTCAAG
AGGGCATTAGCAATAGTGTTTATAAGGCCCCCTTGTTAACCTAAACGGGTAGCATATGCT
TCCCGGGTAGTAGTATATACTATCCAGACTAACCTAATTCAATAGCATATGTTACCCAACG
GGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGCGATATCTCCC
ACCCCATGAGCTGTCACGGTTTTTATTACATGGGGTCAGGATTCCACGAGGGTAGTGAACC
ATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAGGAGCGGGCAGTGAACCTCTCCTGAATC
TTCGCCTGCTTCTTCACTCTCCTTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGT
AAGGTGTATGTGAGGTGCTCGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGG
ACGGGGGGGTTCAAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAAACCCTTG
GGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTTTAACAATAGAAATCCATGG

FIG. 11 cont'd

GGTGGGGACAAGCCGTAAAGACTGGATGTCCATCTCACACGAATTTATGGCTATGGGCAA
CACATAATCCTAGTGCAATATGATACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAA
GACAGGTGAACCATGTTGTTACACTCTATTTGTAACAAGGGGAAAGAGAGTGGACGCCGA
CAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAACGGGGCTCCACGCC
AATGGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAATTTGTGGAGTGGGGGC
ACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACTGTAAAATAAGGGTGTAAATAAC
TTGGCTGATTGTAACCCCGCTAACCACTGCGGTCAAACCACTTGCCACAAAACCACTAAT
GGCACCCCGGGGAATACCTGCATAAGTAGGTGGGCGGGCCAAGATAGGGGCGCGATTGC
TGCGATCTGGAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGG
TCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGGCTAATGTTGCCATGGGTAGCATATA
CTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCC
TAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAA
TTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATC
TATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAATAGA
GATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATATACTACCCAAATATCTG
GATAGCATATGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGG
TAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGT
GTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGC
ATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCAT
ATGCTATCCTCATGATAAGCTGTCAAACATGAGAAATTTCTTGAAGACGAAAGGGCCTCGT
GATACGCCTATTTTTATAGGTTAATGTCATGATAAATGGTTTCTTAGACGTCAGGTGGCA
CTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGT
ATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATG
AGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTTCGGGCATTTTGCCTTCCTGTTTTT
GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTG
GGTTACATCGAACTGGATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTCGCCCCGAAGAAC
GTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGAC
GCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTGGTTGAGTAC
TCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTG
CCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAA
GGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTGCCTTGATCGTTGGGAA
CCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATG
GCAACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGG
CTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTG
CAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTC
AGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCA
TTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTTCATTTTTAA
TTTAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCT
TTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTT
GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCA
GATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAGTCTGTAG
CACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAA
GTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGCGG

FIG. 11 cont'd

CTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGA
GATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGAC
AGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGG
GAAACGCCTGGTATCTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGCGTCGATT
TTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTT
ACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCCTGATT
CTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGA
CCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCT
CTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAA
GCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCT
TTACACTTTATGCTTCCGGCTCGTATGTTGTGTGAATTGTGAGCGGATAACAATTTACAC
AGGAAACAGCTATGACCATGATTACGCCAAGCTCTAGCTAGAGGTCGACCAATTCTCATGT
TTGACAGCTTATCATCGCAGATCCGGGCAACGTTGTTGCCATTGCTGCAGGCGCAGAACT
GGTAGGTATGGC (SEQ ID NO: 13)

**MRT3_PTT3_mouseCD79a_IntracellularCD40 (mouseCD79alpha-mouseIntracellularCD40-
P2A skipping element-T2A skipping element-eGFP Reporter)**

MPGGLEALRALPLLLFLSYACLGPGCQALRVEGGPPSLTVNLGEEARLTCENNGRNPNTWWF
SLQSNITWPPVPLPGPGQGTGQLFFPEVNKNHRGLYWCQVIENNILKRSCGYLRVRNPVPRP
FLDMGEGTKNRIITAEGIILLFCAVVPGLLLFRKRWQNEKFGVDMPPDDYEDENLYEGLNLDDC
SMYEDISRGLQGTYQDVGNLHIGDAQLEKPKKVVKKPKDNEILPPAARRQDPQEMEDYPGHNT
AAPVQETLHGCQPVTQEDGKESRISVQERQVTD SIALRPLVGSGATNFSLLKQAGDVEENPGP
GSGEGRGSLLTCGDVEENPGPMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYG
KLTLKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG
NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRH
NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAAGITLGM
DELYK (SEQ ID NO: 14)

Coding Sequence of MRT4_PTT3_mouseCD79b_IntracellularCD40 (7760 bp)

AGATCTATACATTGAATCAATATTGGCAATTAGCCATATTAGTCATTGGTTATATAGCATAAA
TCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTG
GCTCATGTCCAATATGACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAA
TTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAAT
GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATAATGACGTATGTT
CCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAA
TGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCTACT
TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACA
CCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC AAGTCTCCACCCCATTGACG
TCAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCC
GCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGC
TCGTTTAGTGAACCGTCAGATCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTG
TTGGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACC
CGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGG
ATCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCAC

FIG. 11 cont'd

CGTGGCGGGCGGCAGCGGGTGGCGGTTCGGGGTTGTTTCTGGCGGAGGTGCTGCTGATG
ATGTAATTAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGTGGCAGGCTT
GAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAGCGGGCATTACTTCTGCGCTAAG
ATTGTCAGTTTCCAAAACGAGGAGGATTTGATATTCACCTGGCCCGATCTGGCCATACAC
TTGAGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTCCAAG
TTTAAACGATCTCTAGCGATGGCTACACTGGTTCTCAGCTCAATGCCATGTCATTGGCTTT
TGTTTTTGCTGTTGCTGTTCACTGAGGAGCCAGTGCCTGCTATGACATCCTCCGATTTGCC
CTTGAACCTTCAGGGATCACCTGTCAGCCAAATATGGCAGCACCCACGCTTTGCAGCTAAA
AAGCGCTCCAGCATGGTCAAATTTCACTGTTATACAAACCACAGCGGGGCGATTGACCTGGT
TCAGAAAGAGGGGCGAGCCAAACAGCCTCAAGAATTGGTCAGTGAGGAAGGTGCGATAGTGC
AGACACAAAATGGCTCCGTATATACCCTCACTATCCAAAACATTCAGTACGAAGACAACGG
AATCTATTTTTGTAAGCAGAAGTGCGACAGTGCCAATCACAATGTAACAGATAGCTGTGGC
ACCGAGCTTCTGGTTCTTGGCTTCTCAACACTTGATCAGTTGAAGCGACGAAATACCCTGA
AAGACGGTATTATACTCATTCAAACCTTTGCTCATAATCCTGTTTATAATAGTACCTATCTTCC
TGCTCCTTGATAAGGACGACGGGAAAGCAGGCATGGAAGAAGATCACACCTACGAGGGGT
TGAATATCGACCAGACAGCCACCTACGAAGACATTGTGACTCTCCGAACCGGGGAGGTGA
AATGGAGTGTAGGAGAACACCCCGGTCAAGAAAAGAAAGTGGTCAAGAAGCCAAAGGACA
ATGAGATTCTGCCCCCGCTGCAAGAAGGCAAGATCCCCAGGAGATGGAGGACTATCCAG
GCCACAACACAGCCGCCCCAGTGCAGGAACTCTGCACGGTTGCCAACCTGTTACACAGG
AGGATGGGAAGGAAAGCCGCATCAGTGTCAAGAGCGACAAGTGACAGACTCAATAGCCT
TGCGGCCCTCGTTGGCTCCGGCGCTACCAATTTTAGCCTGTTGAAGCAGGCTGGGGACG
TGGAAGAGAACCCCGGACCAGGAAGTGGCGAGGGAAGGGGGAGTCTTCTTACCTGTGGG
GATGTTGAAGAGAATCCTGGCCCCATGGTATCCAAGGGAGAGGAGCTGTTTACAGGCGTT
GTCCCAATTCTCGTGGAATTGGATGGGGACGTAAATGGTCACAAGTTTAGCGTCTCTGGCG
AGGGCGAGGGTGACGCAACATATGGTAAATTGACTCTGAAGTTTATTTGCACCACAGGTAA
ACTTCCTGTGCCTTGGCCTACACTTGTCACTACTCTGACCTATGGCGTGCAATGTTTCTCC
CGCTACCCTGACCATATGAAACAACACGATTTTTTTAAATCTGCCATGCCCCGAAGGGTATGT
GCAGGAACGGACTATTTTCTTTAAGGACGACGGCAATTATAAACTCGAGCTGAGGTCAAA
TTTGAAGGTGACACCTTGGTCAACAGAATAGAAGTCAAGGGCATTGATTTTAAAGAGGACG
GGAACATACTGGGTCAAAATTGGAGTACAATTATAACTCCCACAACGTTTATATAATGGCT
GATAAGCAGAAAAATGGAATTAAGGTCAACTTCAAATCCGACATAACATAGAGGATGGGT
CAGTACAGCTTGCCGACCATTATCAGCAAAACACTCCTATAGGCGATGGACCCGTGCTTTT
GCCTGACAATCATTATCTGTCTACTCAAAGCGCTCTTTCTAAAGATCCTAACGAAAAACGGG
ATCATATGGTGCTCTTGGAGTTTGTAACTGCCGCCGGCATCACATTGGGAATGGACGAACT
CTATAAATGACCCTCTAGAGGGCCCGTTTCTGTCTAGCAAGCTTGCTAGCGGCCGCTCGAG
GCCGGCAAGGCCGGATCCCCGACCTCGACCTCTGGCTAATAAAGGAAATTTATTTTCATT
GCAATAGTGTGTTGGAATTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATC
ATTTGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCGGACGAAC
TAAACCTGACTACGACATCTCTGCCCTTCTTCGCGGGGCAGTGCATGTAATCCCTTCAGT
TGGTTGGTACAACCTTGCCAACTGGGCCCTGTTCCACATGTGACACGGGGGGGGACCAAAC
ACAAAGGGGTTCTCTGACTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCCAACAC
TGAGTGGCTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATTGCCTT
TATGTGTAACCTTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCCAGGGG
CCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGCCTGTGTAGCTACCGA
TAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTTATAAGGCCCCCTTGTTAACCCCTAA

FIG. 11 cont'd

ACGGGTAGCATATGCTTCCCGGGTAGTAGTATATACTATCCAGACTAACCCTAATTCAATAG
CATATGTTACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGG
AACAGCGATATCTCCACCCCATGAGCTGTCACGGTTTTATTACATGGGGTCAGGATTCC
ACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAGGAGCGGGCAG
TGAACCTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCCTTCGTTTAGCTAATAGAATAACTG
CTGAGTTGTGAACAGTAAGGTGTATGTGAGGTGCTCGAAAACAAGGTTTCAGGTGACGCC
CCCAGAATAAAATTTGGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAAC
CCTCACAAACCCCTTGGGCAATAAATACTAGTGTAGGGGTGACGCCCCCAGAATAAAATTT
GGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAAACCCCT
TGGGCAATAAATACTAGTGTAGGATCCTAGTGAATATGATACTGGGGTTATTAAGATGTGT
CCCAGGCAGGGACCAAGACAGGTGAACCATGTTGTTACACTCTATTTGTAACAAGGGGAAA
GAGAGTGGACGCCGACAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAA
ACGGGGCTCCACGCCAATGGGGCCCATAAACAAAGACAAGTGGCCACTCTTTTTTTTGAAA
TTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACTGTAAA
ATAAGGGTGTAAATAACTTGGCTGATTGTAACCCCGCTAACCACTGCGGTCAAACCACTTGC
CCACAAAACCACTAATGGCACCCCGGGGAATACCTGCATAAGTAGGTGGGCGGGCCAAGA
TAGGGGCGCGATTGCTGCGATCTGGAGGACAAATTACACACACTTGC GCCTGAGCGCCAA
GCACAGGGTTGTTGGTCTCATATTCACGAGGTGCTGAGAGCACGGTGGGCTAATGTTG
CCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGG
GTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGT
AGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAG
CATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCA
TATGCTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAT
ACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATATGCTATC
CTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCT
AATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAAT
CTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCT
GTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTGAAGA
CGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTA
GACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAA
ATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGA
AAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTATTCCCTTTTTTGCGGCATT
TTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGATCAG
TTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT
TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGT
ATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAAT
GACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAG
AATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAAC
GATCGGAGGACCGAAGGAGCTAACCCTTTTTTGACAAACATGGGGGATCATGTAACCTCG
CCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC
GATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAAGTGGCGAACTACTTACTCTA
GCTTCCCGGCAACAATTAAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTG
CGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGG
TCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCT
ACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTG

FIG. 11 cont'd

CCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATT
 TAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCA
 AAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGG
 ATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCCCGC
 TACCAGCGGTGGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAGTGG
 CTTGAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCAC
 TTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAAGTGGCTG
 CTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGTCAAGACGATAGTTACCGGATA
 AGGCGCAGCGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAAC
 GACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGA
 AGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG
 AGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGGTTTCGCCACCTC
 TGAAGTGTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAACGCC
 AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCTGGCCTTTTGTGCTCACATGTTCTTTCC
 TGCCTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAAGTGTGATACCGCT
 CGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAAGCGAGGAAGCGGAAGAGCGCC
 CAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATATGCAGCTGGCACGACA
 GGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC
 ATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAG
 CGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAGCTAGAG
 GTCGACCAATTCTCATGTTTGACAGCTTATCATCGCAGATCCGGGCAACGTTGTTGCCATT
 GCTGCAGGCGCAGAACTGGTAGGTATGGC (SEQ ID NO: 15)

**MRT4_PTT3_mouseCD79b_IntracellularCD40 (mouseCD79beta-mouseIntracellularCD40-
 P2A skipping element-T2A skipping element- eGFP Reporter)**

MATLVLSSMPCHWLLFLLLLFSGEPVPAMTSSDLPLNFQGSQCSQIWQHPRFAAKKRSSMVKF
HCYTNHSGALTWFRKRGSQQPQELVSEEGRIVQTQNGSVYTLTIQNIQYEDNGIYFCKQKCD
ANHNVTDSCTELLVLGFSTLDQLKRRNTLKDGIILQTLIIILFIIVPIFLLLDKDDGKAGMEEDHT
YEGLNIDQTATYEDIVTLRTGEVKWSVGEHPGQEKKVVKPKDNEILPPAARRQDPQEMEDYP
GHNTAAPVQETLHGCQPVTEQDGKESRISVQERQVTDSIALRPLVGSAGTNFSLKQAGDVEE
NPGPGSGEGRGSLLTCGDVEENPGPMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEG
 DATYGKLTCLKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
 FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKV
 NFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAA
 GITLGMDELYK (SEQ ID NO: 16)

Coding Sequence of MRT5_Ptt3_humanCD79a_IntracellularCD40 (7638 bp)

AGATCTATACATTGAATCAATATTGGCAATTAGCCATATTAGTCATTGGTTATATAGCATAAA
 TCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTG
 GCTCATGTCCAATATGACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAA
 TTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAAT
 GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTT
 CCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
 CTGCCCCTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAA
 TGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTACGGGACTTTCCTACT

FIG. 11 cont'd

TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACA
CCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC AAGTCTCCACCCCATTGACG
TCAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCC
GCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGC
TCGTTTAGTGAACCGTCAGATCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTG
TTGGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACC
CGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGG
ATCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCAC
CGTGGCGGGCGGCAGCGGGTGGCGGTGCGGGTTGTTTCTGGCGGAGGTGCTGCTGATG
ATGTAATTAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGTGGCAGGCTT
GAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAGCGGGCATTACTTCTGCGCTAAG
ATTGTCAGTTTCCAAAACGAGGAGGATTTGATATTCACCTGGCCCGATCTGGCCATACAC
TTGAGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCAGGTCCAAG
TTTAAACGGATCTCTAGCGATGCCGGGTGGACCCGGTGTCTTGCAAGCCCTCCCCGCTAC
CATCTTTCTTTTGT TTTCTCCTCTCAGCAGTTTATTTGGGTCCCGGCTGCCAAGCCCTCTGGA
TGCACAAAGTCCCGGCCTCTCTTATGGTATCTCTTGGTGAGGATGCGCACTTTCAGTGTCC
ACATAACTCCTCTAATAATGCTAACGTTACATGGTGGAGGGTCCTTCACGGAAATTATACTT
GGCCTCCAGAGTTTTTTGGGGCCTGGCGAGGATCCAAATGGAACGCTGATTATACAGAACG
TGAATAAATCTCACGGAGGGATCTATGTTTGTGAGTTCAAGAGGGGAATGAATCATATCA
GCAAAGCTGCGGGACTTATTTGCGGGTACGACAACCACCACTAGACCATTCTGGATAT
GGGAGAAGGAACCAAAAATAGAATTATCACAGCTGAGGGCATAATCCTCCTCTTTTGTGCA
GTAGTTCCAGGTACCCTCCTGCTCTTTAGAAAGCGATGGCAAAATGAGAACTCGGGTTGG
ATGCTGGAGATGAATATGAGGATGAGAACTGTACGAAGGTCTGAATCTGGATGATTGTAG
TATGTACGAGGATATTTCAAGAGGTCTGCAAGGTACCTATCAAGATGTGCGCTCCTTGAAT
ATCGGCGACGTACAGCTCGAGAAACCGAAAAAAGTGGCTAAGAAACCACTAATAAAGCTC
CCCACCCCAAACAGGAGCCACAAGAGATAAATTTCCAGATGACCTTCCAGGATCAAACAC
AGCAGCACCAAGTTCAAGAAACACTGCACGGCTGCCAACCTGTTACACAGGAAGACGGGAA
AGAGAGCAGAATTTCCGTCCAAGAACGACAGGGCTCTGGTGCTACTAATTTTTCTCTGCTG
AAACAGGCAGGGGACGTGGAAGAGAACCCTGGCCCCATGGTTTCTAAGGGGGGAAGAAGA
CAACATGGCAATTATCAAGGAATTTATGCGATTTAAGGTGCATATGGAAGGCAGCGTGAAT
GGTCACGAGTTCGAAATAGAAGGAGAAGGCGAAGGTAGGCCATACGAAGGCACGCAGAC
TGCGAAATTGAAGGTGACAAAAGGGGGCCCGCTGCCATTTGCGTGGGATATTTTGAGCCC
CCAATTCATGTACGGCAGTAAAGCTTACGTGAAACACCCGGCTGATATCCCAGACTATCTG
AAGCTTTCTTTTCCGGAGGGCTTTAAGTGGGAGCGGGTTATGAATTTTGAAGATGGTGGAG
TCGTACAGTAACCCAAGATTCCAGTTTGCAGGACGGTGAATTTATATATAAGGTGAAATTG
AGGGGAACGAATTTCCCAAGCGATGGACCTGTAATGCAAAAAAACAATGGGGTGGGAA
GCGTCATCTGAGCGCATGTATCCCAGAGGATGGCGCACTTAAAGGCGAAATTAAGCAAAGA
TTGAAATTGAAGGACGGCGGACACTATGACGCTGAAGTAAAGACTACTTATAAAGCCAAAA
AACC GGTTCCA ACTCCCTGGCGCGTATAACGTGAACATAAAGCTGGACATCACAAGCCATAA
CGAAGACTATACATTGTAGAACAGTATGAACGCGCCGAGGGCCGCCACGTACGGGTGG
TATGGATGAACTCTACAAGTGAAGGGCCCGTTTCTGCTAGCAAGCTTGCTAGCGGCCGCT
CGAGGCCGGCAAGGCCGGATCCCCGACCTCGACCTCTGGCTAATAAAGGAAATTTATTT
TCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGC
AAATCATTTGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCCCGCCCGGA
CGAACTAAACCTGACTACGACATCTCTGCCCTTCTTCGCGGGGCAGTGCATGTAATCCCT

FIG. 11 cont'd

TCAGTTGGTTGGTACAACCTTGCCAACTGGGCCCTGTTCCACATGTGACACGGGGGGGGGAC
CAAACACAAAGGGGTTCTCTGACTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCC
AACACTGAGTGGCTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACATT
GCCTTTATGTGTAACCTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCC
AGGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGGGCCTGTGTAGC
TACCGATAAGCGGACCCCTCAAGAGGGCATTAGCAATAGTGTTTATAAGGCCCCCTTGTTAA
CCCTAAACGGGTAGCATATGCTTCCCGGGTAGTAGTATATACTATCCAGACTAACCCCTAATT
CAATAGCATATGTTACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTC
CTAAGGAACAGCGATATCTCCACCCCATGAGCTGTACGGTTTTATTACATGGGGTCAG
GATTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAGGAGC
GGGCAGTGAACCTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCCTTCGTTTAGCTAATAGAA
TAACTGCTGAGTTGTGAACAGTAAGGTGTATGTGAGGTGCTCGAAAACAAGGTTTCAGGTG
ACGCCCCCAGAATAAAATTTGGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAA
TATAACCCTCACAAACCCCTTGGGCAATAAATACTAGTGTAGGAATGAAACATTCTGAATAT
CTTTAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCATCTCACA
CGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGATACTGGGGTTATTAAGAT
GTGTCCCAGGCAGGGACCAAGACAGGTGAACCATGTTGTTACACTCTATTTGTAACAAGGG
GAAAGAGAGTGGACGCCGACAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAA
TTAAACGGGGCTCCACGCCAATGGGGCCCATAAACAAGACAAGTGGCCACTCTTTTTTTT
GAAATTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACTG
TAAAATAAGGGTGTAACTTGGCTGATTGTAAACCCCGCTAACCACTGCGGTCAAACCAC
TTGCCACAAAACCACTAATGGCACCCCGGGGAATACCTGCATAAGTAGGTGGGCGGGCC
AAGATAGGGGCGCGATTGCTGCGATCTGGAGGACAAATTACACACACTTGCGCCTGAGCG
CCAAGCACAGGGTTGTTGGTCCTCATATTCACGAGGTGCTGAGAGCACGGTGGGCTAAT
GTTGCCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATAT
CTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCT
GGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGG
GTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGT
AGCATATGCTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAG
CATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATATG
CTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCT
ATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATC
CTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTA
ATCTGTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGAGAATTTTCTTG
AAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTT
CTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATA
TTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCTTTTTTTCGG
CATTTTGCCTTCCTGTTTTTGTACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGAT
CAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAG
AGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCG
CGGTATTATCCCGTGTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTC
AGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT
AAGAGAATTATGCAGTGTGCCATAACCATGAGTGATAAACAAGTGGGCCAACTTACTTCTG
ACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTA

FIG. 11 cont'd

ACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGAC
ACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACCTATTAAGTGGCGAACTACTTA
CTCTAGCTTCCCGGCAACAATTAAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACT
TCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCG
TGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGT
TATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGAT
AGGTGCCTCACTGATTAAGCATTGGTAAGTGTGACACCAAGTTTACTCATATATACTTTAGA
TTGATTTAAACTTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCAT
GACCAAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATC
AAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACC
ACCGCTACCAGCGGTGGTTTGTGGCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTA
ACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCC
ACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCACT
GGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACC
GGATAAGGCGCAGCGGTGGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGC
GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTC
CCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCG
CACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGGTTTTGCCA
CCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAA
CGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGTCCTTTTGTGTCACATGTTT
TTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATAC
CGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAG
CGCCCAATACGAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCAC
GACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTC
ACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGT
GAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTAGCTA
GAGGTCGACCAATTCTCATGTTTGACAGCTTATCATCGCAGATCCGGGCAACGTTGTTGCC
ATTGCTGCAGGCGCAGAACTGGTAGGTATGGC (SEQ ID NO: 17)

**MRT5_Ptt3_humanCD79a_IntracellularCD40 (humanCD79alpha-humanIntracellularCD40-
P2A skipping element-mCherry Reporter)**

MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSNNA
NVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQSCGYLR
VRQPPRPFLDMGEGTKNRIITAEGIILLFCVVPGTLLLFRKRWQNEKLGLDAGDEYEDENLYE
GLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKPKKVAKKPTNKAPHPKQEPQEIFPDD
LPGSNTAAPVQETLHGCQPVQTQEDGKESRIESVQERQGGSGATNFSLLKQAGDVEENPGPMVS
KGEEDNMAIIEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTGKGPLPFAWDIL
SPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKL
RGTNFSPDGPVMQKKTMGWEASSERMPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKK
PVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK* (SEQ ID NO: 18)

Coding Sequence of MRT6_Ptt3_humanCD79b_IntracellularCD40 (7647 bp)

AGATCTATACATTGAATCAATATTGGCAATTAGCCATATTAGTCATTGGTTATATAGCATAAA
TCAATATTGGCTATTGGCCATTGCATACGTTGTATCTATATCATAATATGTACATTTATATTG
GCTCATGTCCAATATGACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAA

FIG. 11 cont'd

TTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAAT
GGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTT
CCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAA
TGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTCTTACT
TGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGTATGCGGTTTTTGGCAGTACA
CCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACCCCATTTGACG
TCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAATAACCCC
GCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGC
TCGTTTAGTGAACCGTCAGATCCTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTG
TTGGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACC
CGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGG
ATCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGTCGCAAGGTAGGCTGAGCAC
CGTGGCGGGCGGCAGCGGGTGGCGGTGCGGGTTGTTTCTGGCGGAGGTGCTGCTGATG
ATGTAATTAAGTAGGCGGTCTTGAGACGGCGGATGGTCGAGGTGAGGTGTGGCAGGCTT
GAGATCCAGCTGTTGGGGTGAGTACTCCCTCTCAAAGCGGGCATTACTTCTGCGCTAAG
ATTGTCAGTTTCCAAAACGAGGAGGATTTGATATTCACCTGGCCCGATCTGGCCATACAC
TTGAGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCAGGTCCAAG
TTTAAACGGATCTCTAGCGATGGCCCGACTCGCACTGTCACCAGTACCGTCACATTGGATG
GTGGCATTGTTGCTTCTTCTGAGTGCTGAACCCGTCCCAGCCGCTCGGAGTGAGGATCGG
TACCGGAATCCGAAAGGCTCAGCATGTTCCCGAATCTGGCAATCACCTAGGTTTATTGCTA
GAAAACGCGGCTTCACAGTCAAAATGCACTGCTACATGAACAGCGCAAGTGGTAACGTTAG
CTGGCTTTGGAAGCAGGAGATGGACGAAAACCCGCAACAGCTCAAATTGAAAAGGGACG
CATGGAGGAATCCCAAATGAATCACTGGCAACACTTACGATCCAAGGTATTAGATTCGAG
GATAACGGAATCTACTTCTGTCAGCAGAAATGCAATAATACGAGCGAAGTCTATCAAGGCT
GTGGTACAGAGCTGAGAGTGATGGGGTTTAGCACTCTGGCCCAACTTAAACAAAGAAACA
CCTTGAAGGACGGAATAATCATGATCCAACTTTGCTCATCATTCTTTTATCATAGTGCCT
ATTTTCTTGTTGCTCGATAAGGACGACAGTAAAGCGGGAATGGAAGAAGATCATACATAG
AGGGCCTTGACATTGACCAAACCTGCGACGTATGAAGACATAGTTACCTTGCGGACGGGCG
AAGTTAAATGGAGTGTTGGCGAGCATCCTGGTCAGGAAAAAAGTGGCTAAGAAACCAAC
TAATAAAGCTCCCCACCCCAAACAGGAGCCACAAGAGATAAATTTCCAGATGACCTTCCA
GGATCAAACACAGCAGCACCAGTTCAAGAAACACTGCACGGCTGCCAACCTGTTACACAG
GAAGACGGGAAAGAGAGCAGAATTTCCGTCCAAGAACGACAGGGCTCTGGTGTACTAAT
TTTTCTCTGCTGAAACAGGCAGGGGACGTGCAAGAGAACCCTGGCCCCATGTTTTCTAAG
GGGGAAGAAGACAACATGGCAATTATCAAGGAATTTATGCGATTTAAGGTGCATATGGAAG
GCAGCGTGAATGGTCACGAGTTCGAAATAGAAGGAGAAGGCGAAGGTAGGCCATACGAAG
GCACGCAGACTGCGAAATTGAAGGTGACAAAAGGGGGCCCGCTGCCATTTGCGTGGGATA
TTTTGAGCCCCCAATTCATGTACGGCAGTAAAGCTTACGTGAAACACCCGGCTGATATCCC
AGACTATCTGAAGCTTTCTTTTCCGGAGGGCTTTAAGTGGGAGCGGGTTATGAATTTTGAA
GATGGTGGAGTCGTACAGTAACCCAAGATTCCAGTTTGCAGGACGGTGAATTTATATATA
AGGTGAAATTGAGGGGAACGAATTTCCCAAGCGATGGACCTGTAATGCAAAAAAACAAT
GGGGTGGGAAGCGTCATCTGAGCGCATGTATCCCGAGGATGGCGCACTTAAAGGCGAAAT
TAAGCAAAGATTGAAATTGAAGGACGGCGGACACTATGACGCTGAAGTAAAGACTACTTAT
AAAGCCAAAAAACCAGTCCAACCTCCCTGGCGCGTATAACGTGAACATAAAGCTGGACATCA
CAAGCCATAACGAAGACTATACCATTGTAGAACAGTATGAACGCGCCGAGGGCCGCCACA

FIG. 11 cont'd

GTACGGGTGGTATGGATGAACTCTACAAGTGAAGGGCCCGTTTCTGCTAGCAAGCTTGCT
AGCGGCCGCTCGAGGCCGGCAAGGCCGGATCCCCCGACCTCGACCTCTGGCTAATAAAG
GAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTGTGTCTCTCACTCGGAAGGACATA
TGGGAGGGGCAAATCATTTGGTCGAGATCCCTCGGAGATCTCTAGCTAGAGGATCGATCCC
CGCCCCGGACGAACTAAACCTGACTACGACATCTCTGCCCTTCTTCGCGGGGCGAGTGCA
TGTAATCCCTTCAGTTGGTTGGTACAACCTTGCCAACTGGGCCCTGTTCCACATGTGACACG
GGGGGGGACCAAACACAAAGGGGTTCTCTGACTGTAGTTGACATCCTTATAAATGGATGTG
CACATTTGCCAACACTGAGTGGCTTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCA
ACACAACATTGCCTTTATGTGTAACCTTTGGCTGAAGCTCTTACACCAATGCTGGGGGACA
TGTACCTCCCAGGGGGCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCAGAGGGG
CCTGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTTATAAGGCC
CCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGGTAGTAGTATATACTATCCAGAC
TAACCCTAATTCAATAGCATATGTTACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAG
TAAAAGGGTCCTAAGGAACAGCGATATCTCCACCCCATGAGCTGTCACGGTTTTATTAC
ATGGGGTCAGGATTCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAG
ATCAAGGAGCGGGCAGTGAACCTCTCTGAATCTTCGCCTGCTTCTTCATTCTCCTTCGTTTA
GCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGTGAGGTGCTCGAAAACAAG
GTTTCAGGTGACGCCCCAGAATAAAATTTGGACGGGGGGTTTCAAGTGGTGGCATTGTGCT
ATGACACCAATATAACCCTCACAAACCCTTGGGCAATAAATACTAGTGTAGGAATGAAACA
TTCTGAATATCTTTAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGT
CCATCTCACACGAATTTATGGCTATGGGCAACACATAATCCTAGTGCAATATGATACTGGG
GTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCATGTTGTTACACTCTATT
TGTAACAAGGGGAAAGAGAGTGGACGCCGACAGCAGCGGACTCCACTGGTTGTCTCTAAC
ACCCCCGAAAATTAACGGGGCTCCACGCCAATGGGGGCCATAAACAAGACAAGTGGCC
ACTCTTTTTTTTTGAAATTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCG
GTTTTGGACTGTAAAATAAGGGTGTAAATACTTGGCTGATTGTAACCCCGCTAACCACTGC
GGTCAAACCACTTGCCCAAAAACCACTAATGGCACCCCGGGGAATACCTGCATAAGTAG
GTGGGCGGGCCAAGATAGGGGCGCGATTGCTGCGATCTGGAGGACAAATTACACACACTT
GCGCCTGAGCGCCAAGCACAGGGTTGTTGGTCCTCATATTCACGAGGTGCTGAGAGCAC
GGTGGGCTAATGTTGCCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTAT
CCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCC
TAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAA
TCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCT
GTATCCGGGTAGCATATGCTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTAT
ATCTGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGG
GTAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGT
AGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGC
ATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTAT
ATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTCATGATAAGCTGTCAAACATGA
GAATTTTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTAAATGTCATGAT
AATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT
TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATG
CTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCC
CTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAG
ATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTA

FIG. 11 cont'd

AGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTG
CTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATA
CACTATTCTCAGAATGACTTGTTGAGTACTACCAGTCACAGAAAAGCATCTTACGGATG
GCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAACTGCGGCCAA
CTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGG
GATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGAC
GAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACATTAACTGGC
GAACTACTTACTCTAGCTTCCCGGCAACAATTAAGACTGGATGGAGGCGGATAAAGTTG
CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAG
CCGGTGAGCGTGCGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCC
CGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATAT
ATACTTTAGATTGATTTAAACTTTCATTTTTAATTTAAAGGATCTAGGTGAAGATCCTTTTTG
ATAATCTCATGACCAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTCAGACCCCGTA
GAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAC
AAAAAAACCACCGCTACCAGCGGTGGTTTGTGGCCGGATCAAGAGCTACCAACTCTTTTT
CCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGT
AGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCT
GTTACCACTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAAGCAAGACG
ATAGTTACCGGATAAGGCGCAGCGGTGCGGCTGAACGGGGGGTTCGTGCACACAGCCCA
GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCG
CCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACA
GGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTGCGG
GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCT
ATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGTGCTGGCCTTTTGT
CACATGTTCTTTCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTG
AGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAG
CGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCA
GCTGGCACGACAGGTTTCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGA
GTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGT
GGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCAAGC
TCTAGCTAGAGGTCGACCAATTCTCATGTTTGACAGCTTATCATCGCAGATCCGGGCAACG
TTGTTGCCATTGCTGCAGGCGCAGAACTGGTAGGTATGGC (SEQ ID NO: 19)

MRT6_Ptt3_humanCD79b_IntracellularCD40 (humanCD79beta-humanIntracellularCD40-P2A
skipping element-mCherry Reporter)

MARLALSPVPSHWMVALLLLSAEPVPAARSEDYRNPKGSACSRIWQSPRFIARKRGFTVKM
HCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIYFCQQK
CNNTSEVYQCGTELVRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVIPILLDKDDSKAGMEE
DHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQEKVAKKPTNKAPHPKQEPQEINFPDD
LPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQGSATNFSLLKQAGDVEENPGPMVSK
GEEDNMAIIEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILS
PQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLR

FIG. 11 cont'd

GTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKP
VQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK (SEQ ID NO: 20)

Anti-gp120 scFv - Linker - StrepTagII - Gly6 Linker- full CD40 protein - Gly6 Linker - HA tag

QVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWVRQAPGQRFWMGWINPYNGNKEF
SAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEWGWDDSPYDNYYMDVWGKGT
TVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATFSCRSSHRSRRAVWYQHQP
GQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDFALYYCQVYGASSYTFGQGTKL
ERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPPTACREKQYLINSQCCSLCQPGQKLVS DCT
EFTETECLPCGESEFLDTWNRETHCHQHLYCDPNLGLRVQKGTSETDTICTCEEGWHCTSE
ACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQA
GTNKT DVVCGPQDRLRALVVIPIIFGILFAILLVLVKKVAKKPTNKAPHPKQEPQEINFPDDLPGS
NTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGGGGYPYDVPDYA (SEQ ID NO: 21)

Anti-gp120 scFv - Linker - StrepTagII- Gly6 Linker- IgG Hinge- Gly6 Linker- CD40 Transmembrane Domain - CD40 Intracellular Domain - Gly6 Linker - HA tag

QVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWVRQAPGQRFWMGWINPYNGNKEF
SAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEWGWDDSPYDNYYMDVWGKGT
TVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATFSCRSSHRSRRAVWYQHQP
GQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDFALYYCQVYGASSYTFGQGTKL
ERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPKSCDKTHTCPPCPGGGGGGALVVIPIIFGIL
FAILLVLVKKVAKKPKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQE
DGKESRISVQERQGGGGGGYPYDVPDYA (SEQ ID NO: 22)

Anti-gp120 scFv - Linker - StrepTagII- Gly6 Linker- IgG Hinge- Gly6 Linker- IgG2

Transmembrane Domain - Gly6 Linker - CD40 Intracellular Domain - Gly6 Linker - HA tag
QVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWVRQAPGQRFWMGWINPYNGNKEF
SAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEWGWDDSPYDNYYMDVWGKGT
TVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATFSCRSSHRSRRAVWYQHQP
GQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDFALYYCQVYGASSYTFGQGTKL
ERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPKSCDKTHTCPPCPGLWTTITIFITLFLLSVCY
SATITFFGGGGGGKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQED
GKESRISVQERQGGGGGGYPYDVPDYA (SEQ ID NO: 23)

Anti-gp120 scFv - Linker - StrepTagII - Gly6 Linker- full CD40 protein - Gly6 Linker- FkBP12v36 - GlySer Linker - FkBP12v36- Gly6 Linker - HA tag

QVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWVRQAPGQRFWMGWINPYNGNKEF
SAKFQDRVTFTADTSANTAYMELRSLRSADTAVYYCARVGEWGWDDSPYDNYYMDVWGKGT
TVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATFSCRSSHRSRRAVWYQHQP
GQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDFALYYCQVYGASSYTFGQGTKL
ERKGSAGSAAGSGEFWSHPQFEKGGGGGGEPPTACREKQYLINSQCCSLCQPGQKLVS DCT
EFTETECLPCGESEFLDTWNRETHCHQHLYCDPNLGLRVQKGTSETDTICTCEEGWHCTSE
ACESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQA
GTNKT DVVCGPQDRLRALVVIPIIFGILFAILLVLVKKVAKKPTNKAPHPKQEPQEINFPDDLPGS

FIG. 11 cont'd

NTAAPVQETLHGCQPVTQEDGKESRISVQERQGGGGGGGVQVETISPGDGRTFPKRGQTCV
VHYTGMLLEDGKKVDSSRDNRNPKFKMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGAT
GHPGIIPPHATLVFDVELLKLEGGGGSGGGGSGGGGSGVQVETISPGDGRTFPKRGQTCVVH
YTGMLEDGKKVDSSRDNRNPKFKMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGH
PGIIPPHATLVFDVELLKLEGGGGGGYPYDVPDYAG (SEQ ID NO: 24)

MouseCD79alpha-IntracellularCD40

MPGGLEALRALPLLLFLSYACLGPGCQALRVEGGPPSLTVNLGEEARLTCENNGRNPNTWWF
SLQSNITWPPVPLPGPGQGTGQLFFPEVNKNHRGLYWCQVIENNILKRSCGYLRVRNPVPRP
FLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKFGVDMPDDYEDENLYEGLNLDDC
SMYEDISRGLQGTYQDVGNLHIGDAQLEKPKKVKKPKDNEILPPAARRQDPQEMEDYPGHNT
AAPVQETLHGCQPVTQEDGKESRISVQERQVTD SIALRPLV (SEQ ID NO: 25)

MouseCD79beta-IntracellularCD40

MATLVLSMPCHWLLFLLLLFSGEPVPAMTSSDLPLNFQGSQCSQIWQHPRFAAKKRSSMVKF
HCYTNHSGALTWFRKRGSQQPQELVSEEGRIVQTQNGSVYTLTIQNIQYEDNGIYFCKQKCD
ANHNVTDSCGTELLVLGFSTLDQLKRRNTLKDGIILIQTLIIILFIIVPIFLLLDKDDGKAGMEEDHT
YEGLNIDQTATYEDIVTLRTGEVKWSVGEHPGQEKVKVKKPKDNEILPPAARRQDPQEMEDYP
GHNTAAPVQETLHGCQPVTQEDGKESRISVQERQVTD SIALRPLV (SEQ ID NO: 26)

HumanCD79alpha-IntracellularCD40

MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSNNA
NVTWWRVLHGNYTWPEFLGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQSCGYLR
VRQPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGDEYEDENLYE
GLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKPKKVAKKPTNKAPHPKQEPQEINFPDD
LPGSNTAAPVQETLHGCQPVTQEDGKESRIESVQERQ (SEQ ID NO: 27)

HumanCD79beta-IntracellularCD40

MARLALSPVPSHWMVALLLLSAEPVPAARSEDYRNPKGSACSRIWQSPRFIARKRGFTVKM
HCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIYFCQQK
CNNTSEVYQCGGTELRLVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFLLLDKDDSKAGMEE
DHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQEKVAKKPTNKAPHPKQEPQEINFPDD
LPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ (SEQ ID NO: 28)

Human CD40 signal peptide

MVRLPLQCVLWGCLLTAVHP (SEQ ID NO: 29)

Anti-gp120 scFv

QVQLVQSGAEVKKPGASVKVSCQASGYRFSHFTVHWVRQAPGQRFQWGMWINPYNGNKEF
SAKFQDRVFTADTSANTAYMELRSLRSADTAVYYCARVGEWGWDDSPYDNYMDVWGKGT
TVIVSSGGGGSGGGGSGGGGSEIVLTQAPGTLSPGERATFSCRSSHRSRRAVWYQHQP
GQAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDFALYYCQVYGASSYTFGQGTKL
ERKGSAGSAAGSGEF (SEQ ID NO: 30)

FIG. 11 cont'd

Full CD40 protein

EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHXY
CDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHRSCSPGFGVKQIATGVSDTICEP
CPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCQPQDRLRALVVIPIIFGILFAILL
VLVKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE
RQ (SEQ ID NO: 31)

Extracellular CD40 protein

EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHXY
CDPNLGLRVQQKGTSETDTICTCEEGWHCTSEACESCVLHRSCSPGFGVKQIATGVSDTICEP
CPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCQPQDRLR (SEQ ID NO: 32)

CD40 Transmembrane Domain

ALVVIPIIFGILFAILLVLV (SEQ ID NO: 33)

CD40 Intracellular Domain

KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
(SEQ ID NO: 34)

Human Intracellular CD40 (from MRT5)

KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRIESVQERQ
(SEQ ID NO: 35)

Mouse Intracellular CD40 (from MRT3 and MRT4)

KKVVKKPKDNEILPPAARRQDPQEMEDYPGHNTAAPVQETLHGCQPVTQEDGKESRISVQER
QVTDSIALRPLV (SEQ ID NO: 36)

IgG hinge

EPKSCDKTHTCPPCP (SEQ ID NO: 37)

IgG2 Transmembrane domain

GLWTTITIFITLFLLSVCYSATITFF (SEQ ID NO: 38)

FkBP12v36

GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFFKMLGKQEVIRGWEE
GVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 39)

FKBP12 domain

MGVQVETISPGDGRTFPKRGQTCWHYTGMLEDGKKFDSSRDRNPFKMLGKQEVIRGWEEG
VAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 40)

FRB segment of mTOR

MASRILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRD
LMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRISKLES (SEQ ID NO: 41)

FIG. 11 cont'd

mCherry reporter

MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFA
WDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIY
KVKLRGTNFPSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYK
AKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK (SEQ ID NO: 42)

eGFP Reporter

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTT
LTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELK
GIDFKEDGNILGHKLEYNNSHNVIYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGD
GPVLLPDNHVLTQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYK (SEQ ID NO: 43)

mouseCD79alpha

MPGGLEALRALPLLLFLSYACLGPGCQALRVEGGPPSLTVNLGEEARLTCENNGRNPNTWWF
SLQSNITWPPVPLGPGQGTGQLFFPEVNKNHRGLYWCQVIENNILKRSCGYLRVRNPVPRP
FLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFKRWRQNEKFGVDMPPDDYEDENLYEGLNLDDC
SMYEDISRGLQGTYQDVGNLHIGDAQLEKP (SEQ ID NO: 44)

mouseCD79beta

MATLVLSMPCHWLLFLLLLFSGEPVPAMTSSDLPLNFQGSQCSQIWQHPRFAAKKRSSMVKF
HCYTNHSGALTWFRKRGSQQPQELVSEEGRIVQTQNGSVYTLTIQNIQYEDNGIYFCQKQKDS
ANHNVTDSCGTELLVLGFSTLDQLKRRNTLKDGIILIQTLIILFIIVPIFLLLDKDDGKAGMEEDHT
YEGLNIDQTATYEDIVTLRTGEVKWSVGEHPGQE (SEQ ID NO: 45)

humanCD79alpha

MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSNNA
NVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKS HGGIYVCRVQEGNESYQQSCGYLR
VRQPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFKRWRQNEKLGLDAGDEYEDENLYE
GLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP (SEQ ID NO: 46)

humanCD79beta

MARLALSPVPSHWMVALLLLLSAEPVPAARSEDYRNPKGSACSRIWQSPRFIARKRGFTVKM
HCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIYFCQQK
CNNTSEVYQGCGETLRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFLLLDKDDSKAGMEE
DHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQE (SEQ ID NO: 47)

Linker

GGGGGG (SEQ ID NO: 48)

Linker

GGGGSGGGSGGGGS (SEQ ID NO: 49)

StrepTagII

WSHPQFEK (SEQ ID NO: 50)

FIG. 11 cont'd

HA tag

YPYDVPDYA (SEQ ID NO: 51)

His tag

HHHHHH (SEQ ID NO: 52)

Avi tag

GLNDIFEAQKIEWHE (SEQ ID NO: 53)

sFLAG

DYKDE (SEQ ID NO: 54)

Tev

ENLYFQGGS (SEQ ID NO: 55)

Flag tag

DYKDDDDK (SEQ ID NO: 56)

Xpress tag

DLYDDDDK (SEQ ID NO: 57)

Calmodulin tag

KRRWKKNFIAVSAANRFKKISSSGAL (SEQ ID NO: 58)

Myc tag

EQKLISEEDL (SEQ ID NO: 59)

Softag 1

SLAELLNAGLGGS (SEQ ID NO: 60)

Softag 3

TQDPSRVG (SEQ ID NO: 61)

STREP® tag

WRHPQFGG (SEQ ID NO: 62)

V5 tag

GKPIPPLLGLDST (SEQ ID NO: 63)