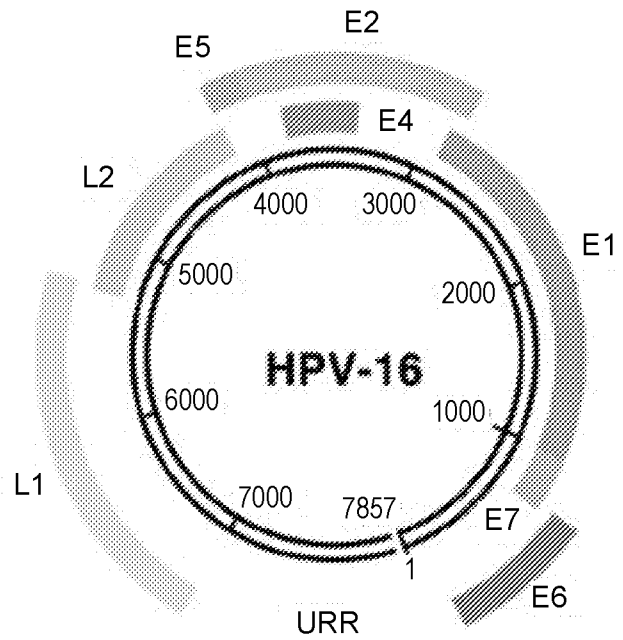

Gene	Function
L1	Major capsid protein.
L2	Minor capsid protein.
E1	Transcription factor, helicase activity. Mediates episomal DNA replication.
E2	Transcription factor. Regulates viral copy number.
E4	Facilitates virion release.
E5	Stimulates cell proliferation and prevents differentiation. Downregulates surface MHC class I expression.
E6	Deregulates cell cycle control through p53 inactivation/ degradation. Induces malignant transformation together with E7.
E7	Keeps cells active in the cell cycle through Rb inactivation. Induces malignant transformation alone and together with E6.

FIG. 1

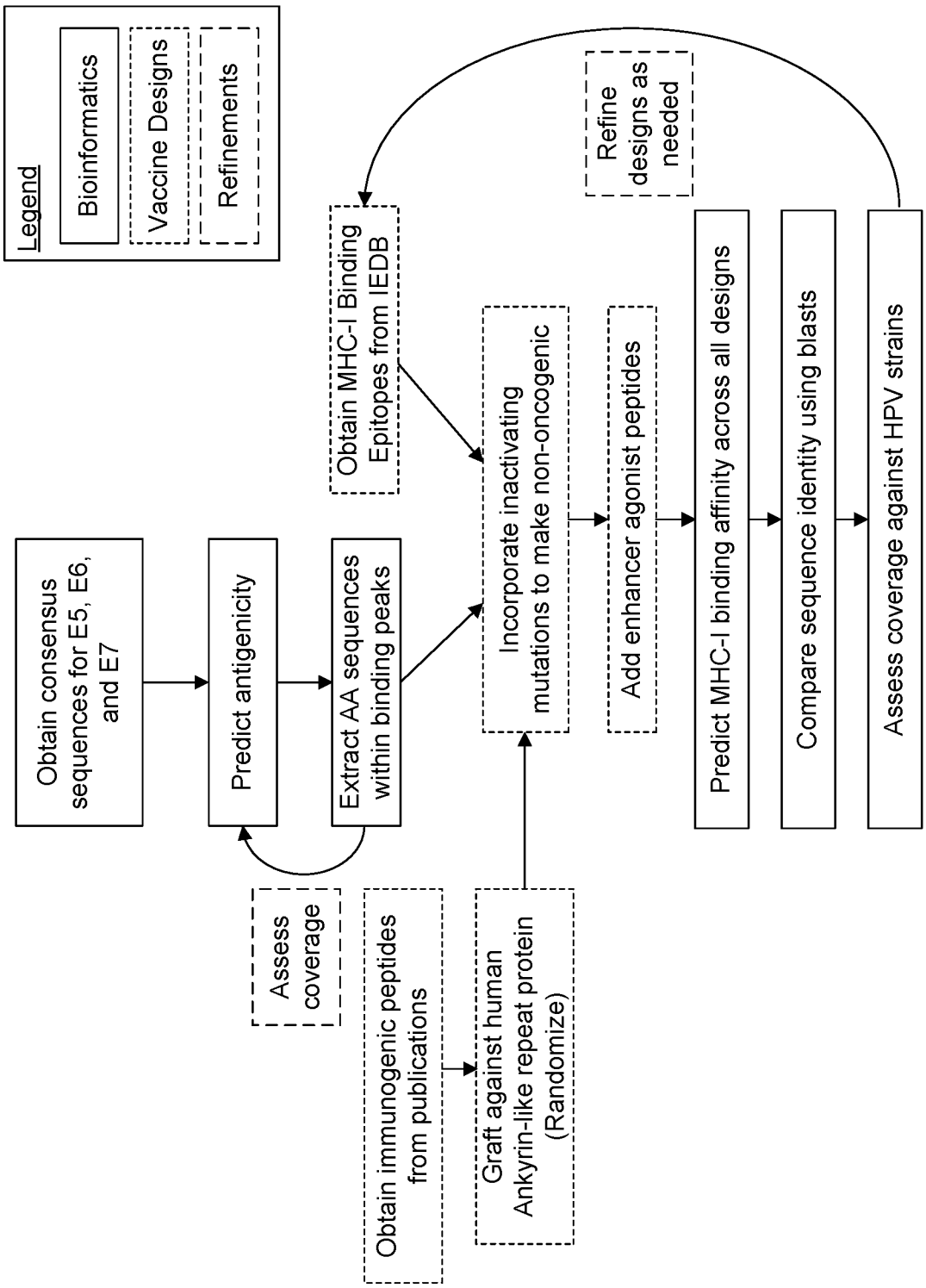
Legend
Bioinformatics
Vaccine Designs
Refinements
Obtain consensus sequences for E5, E6, and E7
Predict antigenicity
Extract AA sequences within binding peaks
Obtain MHC-I Binding Epitopes from IEDB
Incorporate inactivating mutations to make non-oncogenic
Add enhancer agonist peptides
Predict MHC-I binding affinity across all designs
Compare sequence identity using blasts
Assess coverage against HPV strains
Refine designs as needed
Assess coverage
Obtain immunogenic peptides from publications
Graft against human Ankyrin-like repeat protein (Randomize)

FIG. 2

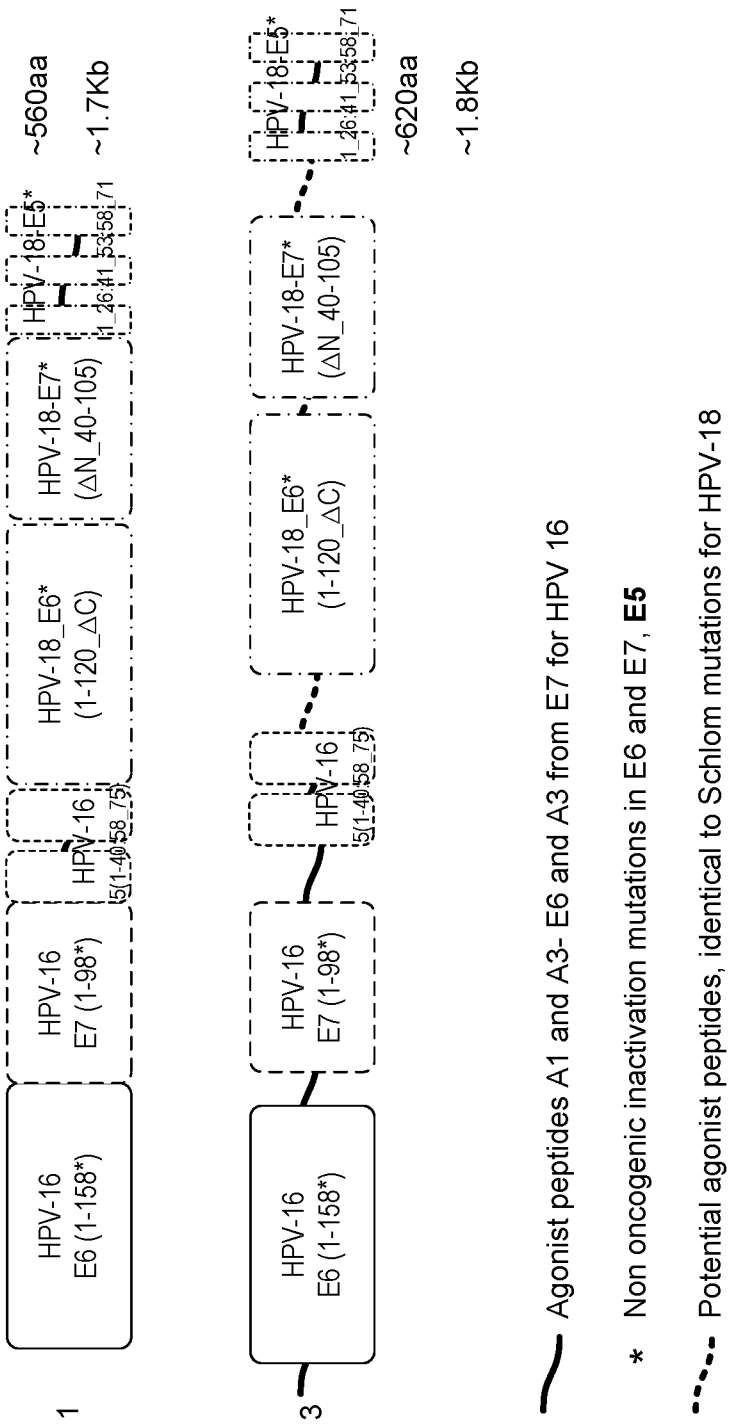
Design 1
HPV-16 E6 (1-158*)
HPV-16 E7 (1-98*)
HPV-16 5(1-40_58_75)
HPV-18_E6* (1-120_ΔC)
HPV-18-E7* (ΔN_40-105)
HPV-18-E5* 1_26_41_53_58_71
~560aa
~1.7Kb
Design 3
HPV-16 E6 (1-158*)
HPV-16 E7 (1-98*)
HPV-16 5(1-40_58_75)
HPV-18_E6* (1-120_ΔC)
HPV-18-E7* (ΔN_40-105)
HPV-18-E5* 1_26_41_53_58_71
~620aa
~1.8Kb
Agonist peptides A1 and A3- E6 and A3 from E7 for HPV 16
* Non oncogenic inactivation mutations in E6 and E7, E5
Potential agonist peptides, identical to Schlom mutations for HPV-18

FIG. 3

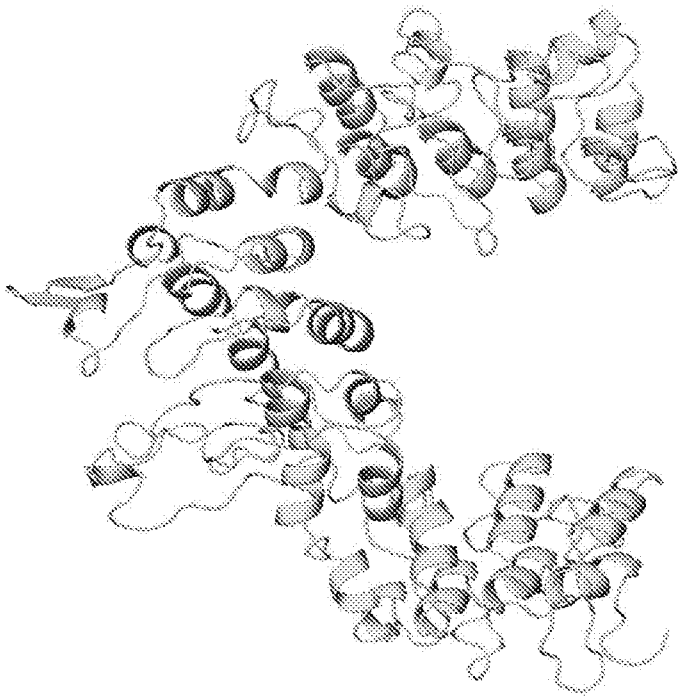

FIG. 4B

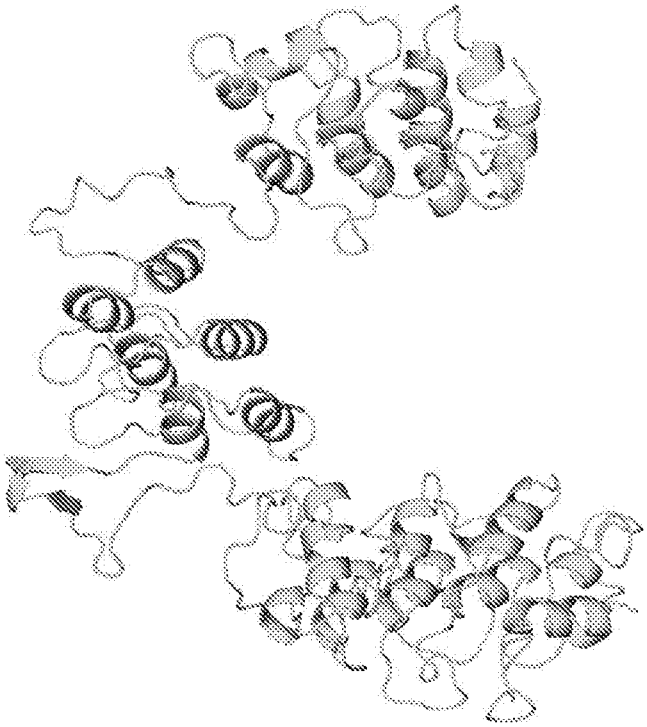

FIG. 4A

```
Score          Expect  Method                      Identities      Positives       Gaps
585 bits(1507) 0.0     Compositional matrix adjust. 365/566(64%)  379/566(66%)  134/566(23%)

Query  4    RELTEVFEFAARYSRSDYIIFVYIPLADAKLPQLCTEVDNMLLMGTLGIVPLHAAVSADT  63
            +ELTEVFEFA +Y     IIFVYIPL   KLPQLCTEV   LLMGTLGIV         T
Sbjct  2    KELTEVFEFAKKY-----IIFVYIPLK-KKLPQLCTEVKK-LLMGTLGIV-------KKT  47

Query  64   LHEYMLDLRNRATDLSEEENDEIDGVNHQHLPARRMHDNLLIRCLRCPLILAARLAVLFL  123
            LHEYMLDL+      SEEENDEIDGVNHQHLPARR   NLLIRCLRC          LFL
Sbjct  48   LHEYMLDLKK-----SEEENDEIDGVNHQHLPARR-KKNLLIRCLRC--------KKLFL  93

Query  124  NTLSFVCPWCASSHADVKCIDFYSRIDDLKLPDLCTELALHWAAAVNNVQLFLNTLSFVG  183
            NTLSFVCPWCAS     KCIDFYSRI   KLPDLCTEL           QLFLNTLSFV
Sbjct  94   NTLSFVCPWCASKK---KCIDFYSRIKK-KLPDLCTEL---------KKQLFLNTLSFV-  139

Query  184  ANKFQQLFLNTLNNRVYDFAFRDLPLFLAAREGSYEKLTNTGLYNLANRLCIVYRDGNPY  243
              KFQQLFLNTL  +VYDFAFRDL           +KLTNTGLYNL  +LCIVYRDGNPY
Sbjct  140  -KKFQQLFLNTL-KKVYDFAFRDLK----------KKLTNTGLYNL-KKLCIVYRDGNPY  186

Query  244  AVCDDHMYMLDLQPETTPRDFAFRDLCIVYIVRLLDLEVSQTSKLTRQTDRTLGIVCPIA  303
            AVCD   YMLDLQPETT + FAFRDLCIVY                    +TLGIVCPI
Sbjct  187  AVCDKK-YMLDLQPETT-KKFAFRDLCIVY-------------------KKTLGIVCPIK  225

Query  304  ARYSRSDRTLEDLLMGVADATIHDIILECVDNMHLDKKQRFHNIPLHAAVSADTTLEQQY  363
                   RTLEDLLMGV   TIHDIILECV   HLDKKQRFHNI         TTLEQQY
Sbjct  226  K------RTLEDLLMGVK-KTIHDIILECVKK-HLDKKQRFHNI-------KKTTLEQQY  270

Query  364  NKPLCDLLRNRATDLCDSTLRLCVMHDRWTGRCMSCCPLILAARLAVSLQDIEITCVSHA  423
            NKPLCDLL+      CDSTLRLCV   RWTGRCMSCC          SLQDIEITCV
Sbjct  271  NKPLCDLLKK-----CDSTLRLCV-KKRWTGRCMSCC--------KKSLQDIEITCVKK-  315

Query  424  DVLCVQSTHVDIDDLAHYNIVTFCCLHWAAAVNNVDDLRAFQQLFLNTLSGANKISEYRH  483
              LCVQSTHVDI   AHYNIVTFCC          DDLRAFQQLFLNTLS   KISEYRH
Sbjct  316  --LCVQSTHVDIKK-AHYNIVTFCC--------KKDDLRAFQQLFLNTLS--KKISEYRH  362

Query  484  YCYNNRKLPQLCTELPLFLAAREGSYEKTVLELTEVANRRTLEDLLMGTDHMFAFKDLFV  543
            YCY  +KLPQLCTEL           +KTVLELTEV  +RTLEDLLMGT   FAFKDLFV
Sbjct  363  YCY-KKKLPQLCTELK----------KKTVLELTEV-KKRTLEDLLMGTKK-FAFKDLFV  409

Query  544  VPRDQLYNKPLCDVIVRTLQDIVLHL  569
            V + QLYNKPLCDV  +TLQDIVLHL
Sbjct  410  V-KKQLYNKPLCDV-KKTLQDIVLHL  433
```

FIG. 5A

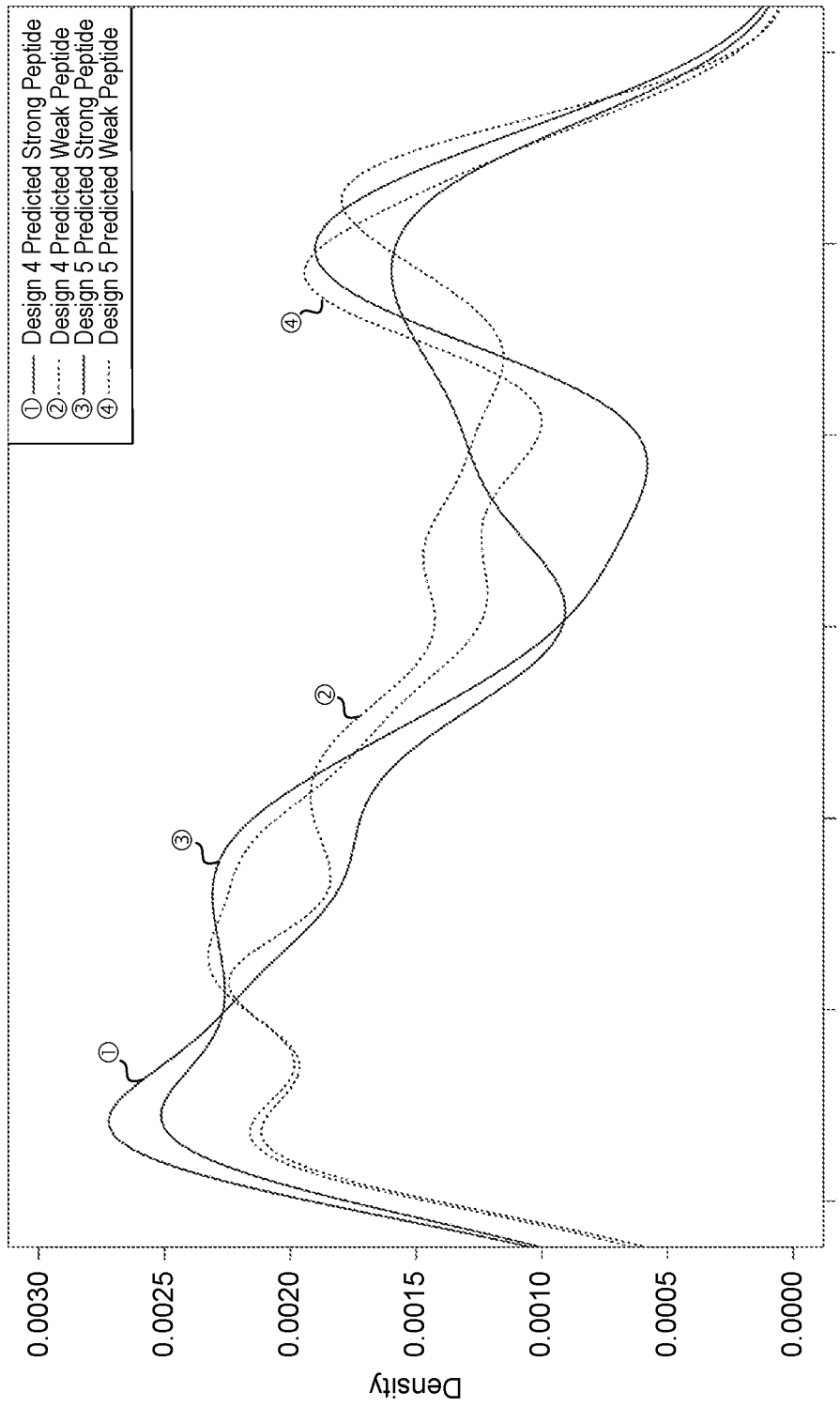
Design 2 Location of Binding Affinities
① Design 4 Predicted Strong Peptide
② Design 4 Predicted Weak Peptide
③ Design 5 Predicted Strong Peptide
④ Design 5 Predicted Weak Peptide
Density
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
0
100
200
300
400
500
600
Position

FIG. 5B

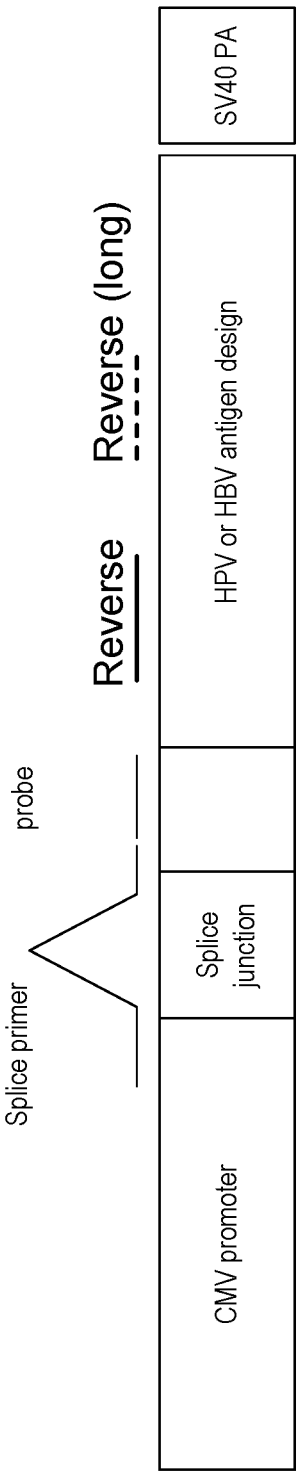
SV40 PA
HPV or HBV antigen design
Reverse (long)
Reverse
probe
Splice primer
Splice junction
CMV promoter

FIG. 6

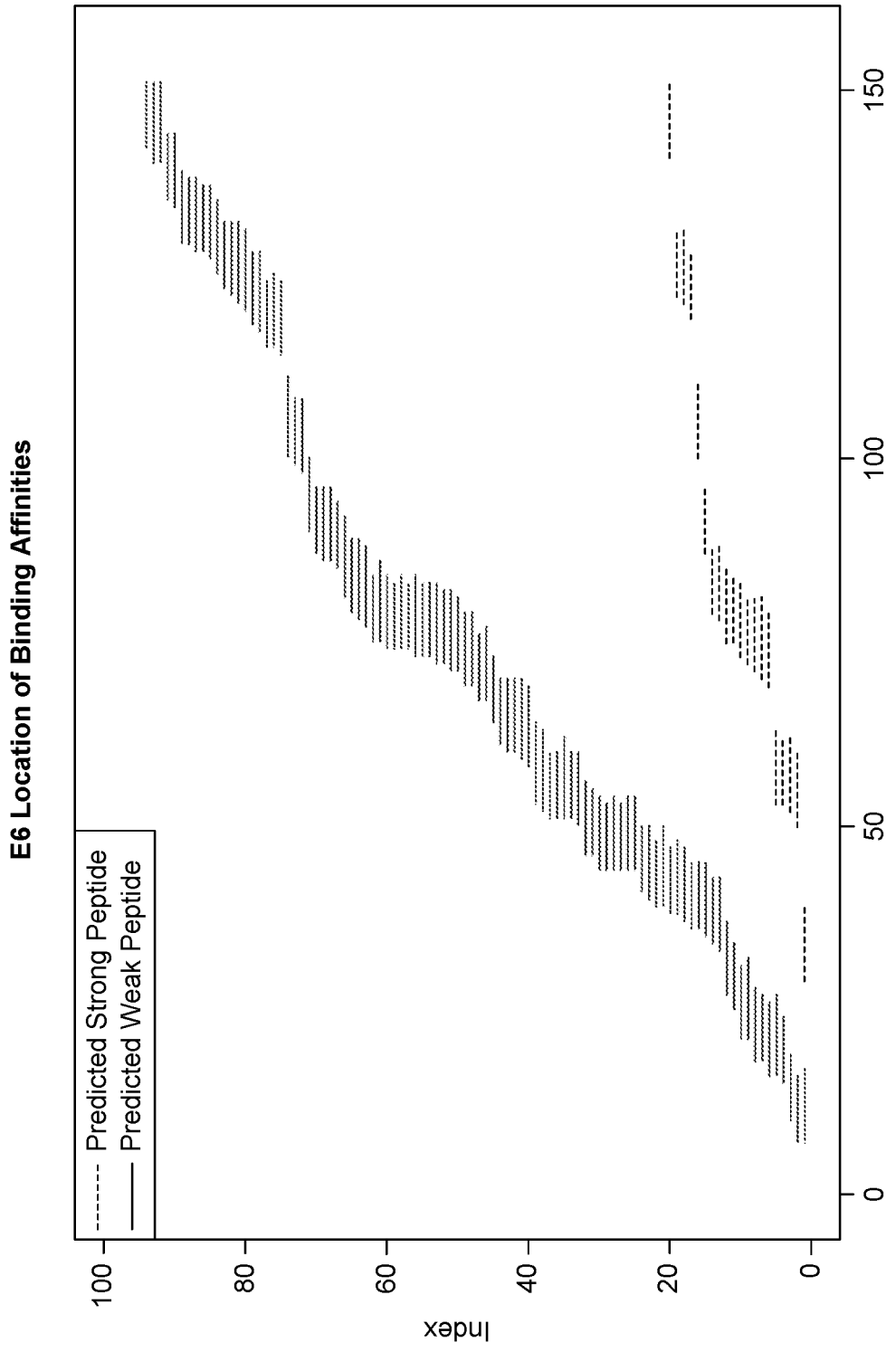
E6 Location of Binding Affinities
Predicted Strong Peptide
Predicted Weak Peptide
Index
Position
0
20
40
60
80
100
0
50
100
150

FIG. 7A

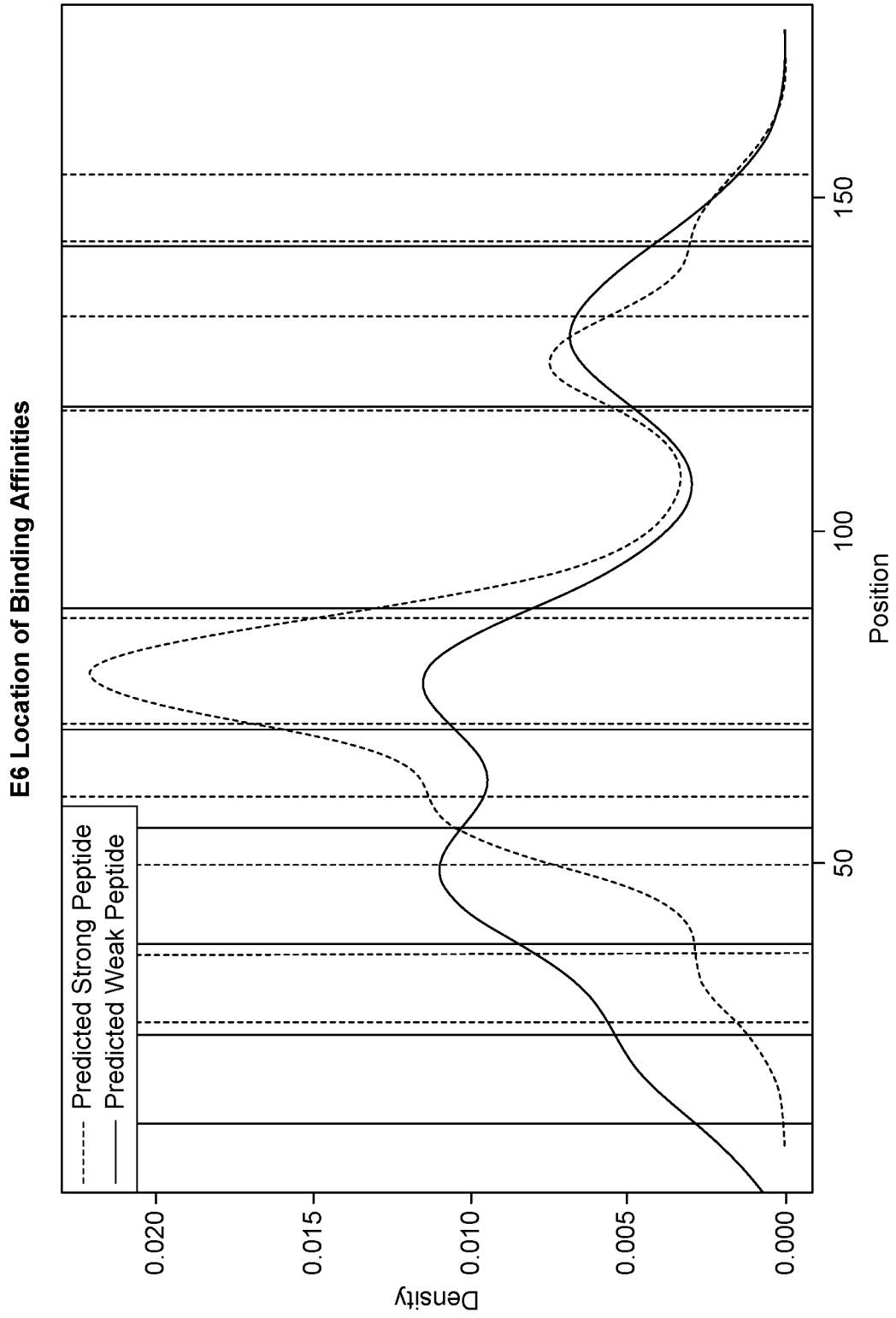
E6 Location of Binding Affinities
Predicted Strong Peptide
Predicted Weak Peptide
Density
0.020
0.015
0.010
0.005
0.000
50
100
150
Position

FIG. 7B

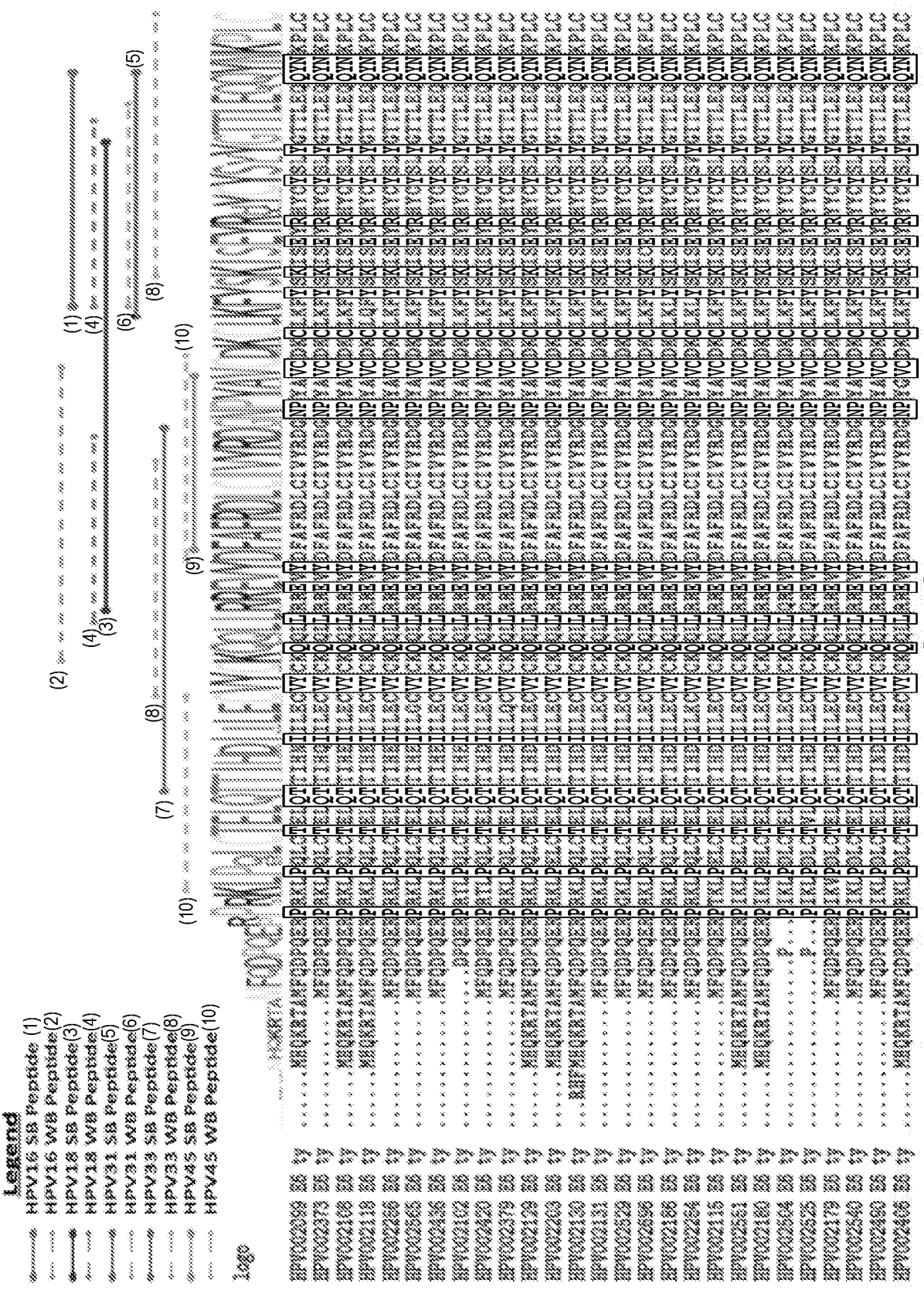
Legend
HPV16 SB Peptide (1)
HPV16 WB Peptide(2)
HPV18 SB Peptide(3)
HPV18 WB Peptide(4)
HPV31 SB Peptide(5)
HPV31 WB Peptide(6)
HPV33 SB Peptide(7)
HPV33 WB Peptide(8)
HPV45 SB Peptide(9)
HPV45 WB Peptide(10)
Logo

FIG. 7C

(MOD-1755822) HPV 16 18 E6 E7 E5 ---------MFQDPQERPRKLPQLCTALQTTIHDIILECVYCKQQLLRREVYDFAFRDGC
(MOD-1755825) HPV 16 18 E6 E7 E5 **klpqlctev**MFQDPQERPRKLPQLCTALQTTIHDIILECVYCKQQLLRREVYDFAFRDGC

(MOD-1755822) HPV 16 18 E6 E7 E5 IVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLC
(MOD-1755825) HPV 16 18 E6 E7 E5 IVYRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLC

(MOD-1755822) HPV 16 18 E6 E7 E5 PEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRAAAA----------MPGDTPTLHE
(MOD-1755825) HPV 16 18 E6 E7 E5 PEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRAAAA**qlynkplcdv**MPGDTPTLHE

(MOD-1755822) HPV 16 18 E6 E7 E5 YMLDLQPETTDLYGYEQLNDSSEEEDEIDGPAGQAAPDRAHYNIVTFCCKCDSTLRRCVQ
(MOD-1755825) HPV 16 18 E6 E7 E5 YMLDLQPETTDLYGYEQLNDSSEEEDEIDGPAGQAAPDRAHYNIVTFCCKCDSTLRRCVQ

(MOD-1755822) HPV 16 18 E6 E7 E5 STHVDIRTLEDLLMGTLGIVCPICSQKP----------MTNLDTASTTLLACFLLCFCVL
(MOD-1755825) HPV 16 18 E6 E7 E5 STHVDIRTLEDLLMGTLGIVCPICSQKP**rtledllmgv**MTNLDTASTTLLACFLLCFCVL

(MOD-1755822) HPV 16 18 E6 E7 E5 LCVCLLIRPLLLSVSTYTRCFIVYIIFVYIPLFLIH---------MARFEDPTRRPYKLP
(MOD-1755825) HPV 16 18 E6 E7 E5 LCVCLLIRPLLLSVSTYTRCFIVYIIFVYIPLFLIH**klpdlctev**MARFEDPTRRPYKLP

(MOD-1755822) HPV 16 18 E6 E7 E5 DLCTALNTSLQDIEITCVYCKTVLELTEVFEFAFKDGFVVYRDSIPHAACHKCIDFYSRI
(MOD-1755825) HPV 16 18 E6 E7 E5 DLCTALNTSLQDIEITCVYCKTVLELTEVFEFAFKDGFVVYRDSIPHAACHKCIDFYSRI

(MOD-1755822) HPV 16 18 E6 E7 E5 RELRHYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRH----------EIDGV
(MOD-1755825) HPV 16 18 E6 E7 E5 RELRHYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRH**kltntglynv**EIDGV

(MOD-1755822) HPV 16 18 E6 E7 E5 NHQHLPARRAAPQRHTMLCMCCKCEARIERVVESSADDLRAFQQLFLNTLSFVCPWCASQ
(MOD-1755825) HPV 16 18 E6 E7 E5 NHQHLPARRAAPQRHTMLCMCCKCEARIERVVESSADDLRAFQQLFLNTLSFVCPWCASQ

(MOD-1755822) HPV 16 18 E6 E7 E5 Q----------MLSLIFLFCFCVCMYVCCHVPLLPSVVVITSPATAFTVYLLPMLLLHIH
(MOD-1755825) HPV 16 18 E6 E7 E5 Q**rafqqlflnv**MLSLIFLFCFCVCMYVCCHVPLLPSVVVITSPATAFTVYLLPMLLLHIH

(MOD-1755822) HPV 16 18 E6 E7 E5 AILS
(MOD-1755825) HPV 16 18 E6 E7 E5 AILS

FIG. 8

(MOD-1755804) HPV- 16 E6(1-151) **-------**MFQDPQERPRKLPQLCT**a**LQTTIHDIILECVYCKQQLLRREVYDFAFRD**g**CIV
native seq **mhqkrta**MFQDPQERPRKLPQLCT**e**LQTTIHDIILECVYCKQQLLRREVYDFAFRD**l**CIV

(MOD-1755804) HPV- 16 E6(1-151) YRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLCPE
native seq YRDGNPYAVCDKCLKFYSKISEYRHYCYSLYGTTLEQQYNKPLCDLLIRCINCQKPLCPE

(MOD-1755804) HPV- 16 E6(1-151) EKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRR**aaaa**
native seq EKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTR**etql**

FIG. 9A

(MOD-1755805) HPV- 16 E7(1-98) n MpGDTPTLHEYMLDLQPETTDLYgYEQLNDSSEEEDEIDGPAGQAaPDRAHYNIVTFCCK
native seq MhGDTPTLHEYMLDLQPETTDLYcYEQLNDSSEEEDEIDGPAGQAePDRAHYNIVTFCCK

(MOD-1755805) HPV- 16 E7(1-98) n CDSTLRrCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP
native seq CDSTLRlCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP

FIG. 9B

(MOD-1755806) HPV- 16 E5(1-40:58 MTNLDTASTTLLACFLLCFCVLLCVCLLIRPLLLSVSTYT----------RCF
native seq MTNLDTASTTLLACFLLCFCVLLCVCLLIRPLLLSVSTYT**sliilvlllwitaasaf**RCF

(MOD-1755806) HPV- 16 E5(1-40:58 IVYIIFVYIPLFLIH--------
native seq IVYIIFVYIPLFLIH**tharflit**

FIG. 9C

```
(MOD-1755807) HPV- 18 E6(1-120)  MARFEDPTRRPYKLPDLCTaLNTSLQDIEITCVYCKTVLELTEVFEFAFKDgFVVYRDSI
seq                              MARFEDPTRRPYKLPDLCTeLNTSLQDIEITCVYCKTVLELTEVFEFAFKDlFVVYRDSI

(MOD-1755807) HPV- 18 E6(1-120)  PHAACHKCIDFYSRIRELRHYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRH
seq                              PHAACHKCIDFYSRIRELRHYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRH

(MOD-1755807) HPV- 18 E6(1-120)  ------------------------------
seq                              lnekrrfhniaghyrgqchsccnrarqerlqrrretqv
```

FIG. 10A

(MOD-1755808) HPV- 18 E7(40-105) ----------EIDGVNHQHLPARRAaPQRHT
native E7 seq **mhgpkatlqdivlhlepqneipvdllcheqlsdseeend**EIDGVNHQHLPARRAePQRHT

(MOD-1755808) HPV- 18 E7(40-105) MLCMCCKCEARIEKVVESSADDLRAFQQLFLNTLSFVCPWCASQQ
native E7 seq MLCMCCKCEARIKLVVESSADDLRAFQQLFLNTLSFVCPWCASQQ

FIG. 10B

(MOD-1755809) HPV- 18 E5(1-26:41 MLSLIFLFCFCVCMYVCCHVPLLPSV----------VVITSPATAFTVY----LLP
native seq MLSLIFLFCFCVCMYVCCHVPLLPSV**cmcayawvlvfvyi**VVITSPATAFTVY**vfcf**LLP

(MOD-1755809) HPV- 18 E5(1-26:41 MLLLHIHAILS--
native seq MLLLHIHAIL**Slq**

FIG. 10C

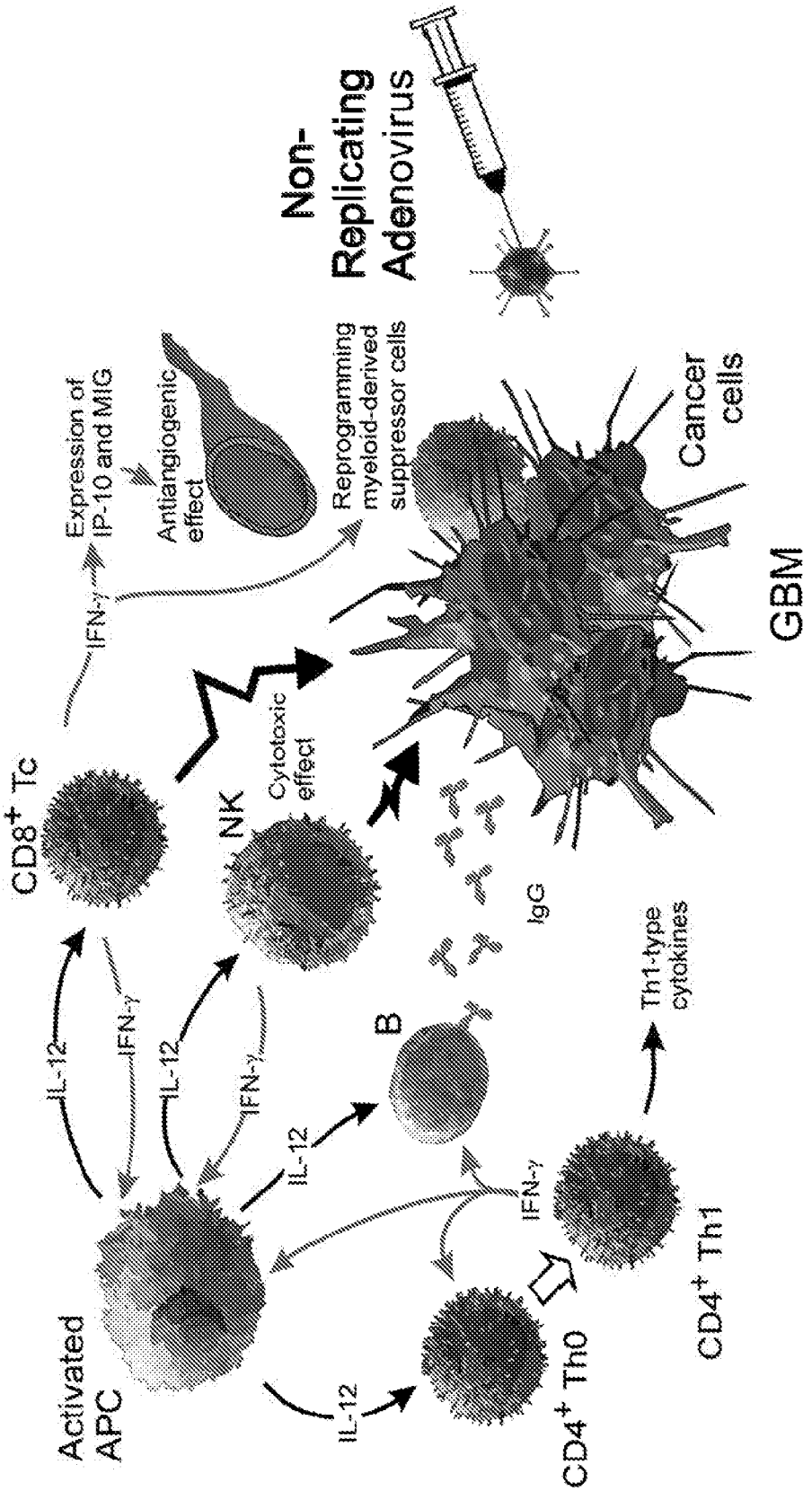
Non-
Replicating
Adenovirus
Expression of
IP-10 and MIG
Antiangiogenic
effect
Reprogramming
myeloid-derived
suppressor cells
Cancer
cells
GBM
IFN-γ
CD8+ Tc
Cytotoxic
effect
NK
IgG
IL-12
IFN-γ
IL-12
IFN-γ
B
IL-12
Th1-type
cytokines
IFN-γ
Activated
APC
IL-12
CD4+ Th0
CD4+ Th1

FIG. 11

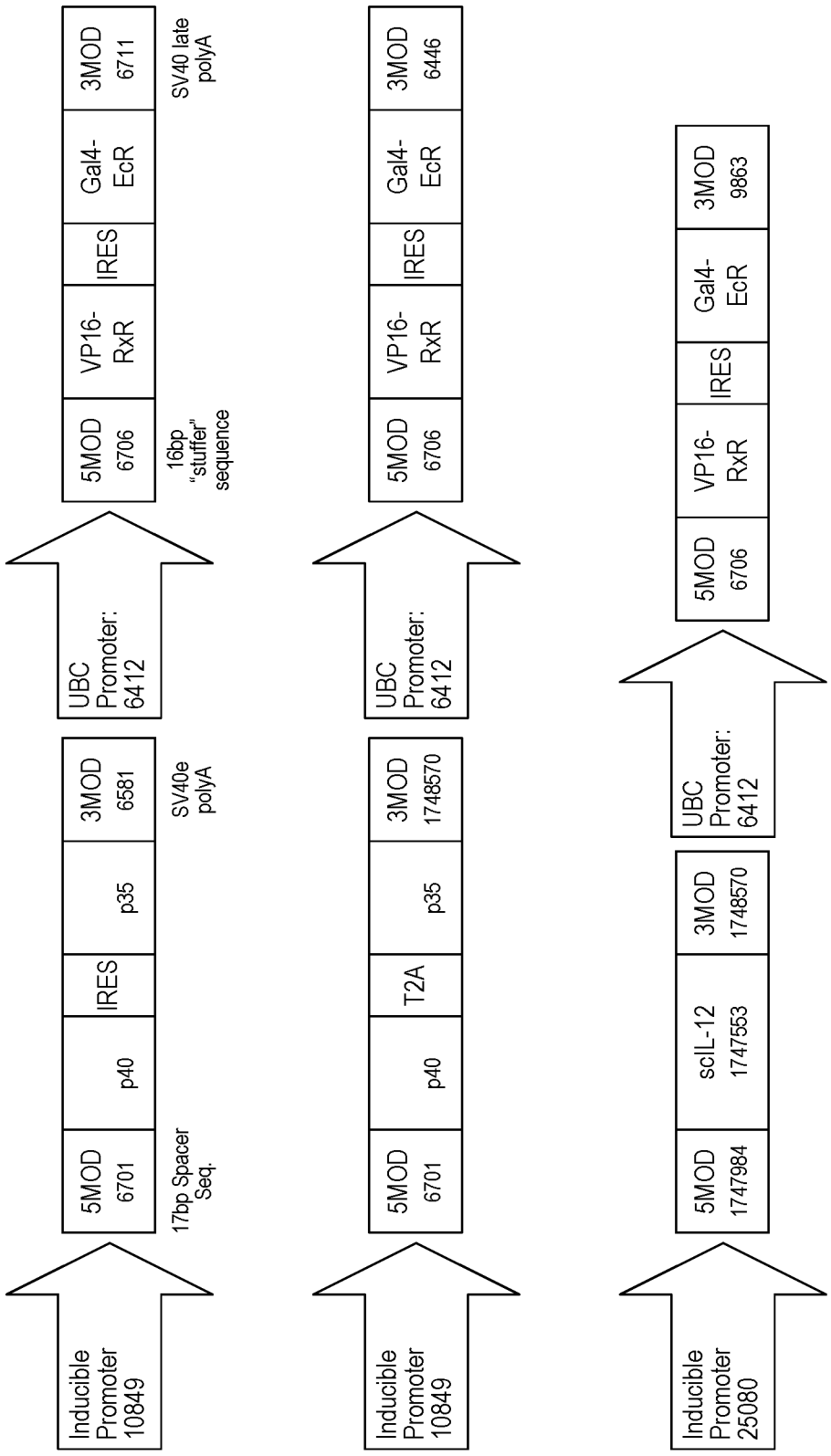
Inducible Promoter 10849
5MOD 6701
p40
IRES
p35
3MOD 6581
SV40e polyA
17bp Spacer Seq.
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 6711
SV40 late polyA
16bp "stuffer" sequence
Inducible Promoter 10849
5MOD 6701
p40
T2A
p35
3MOD 1748570
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 6446
Inducible Promoter 25080
5MOD 1747984
scIL-12 1747553
3MOD 1748570
UBC Promoter: 6412
5MOD 6706
VP16-RxR
IRES
Gal4-EcR
3MOD 9863

FIG. 12