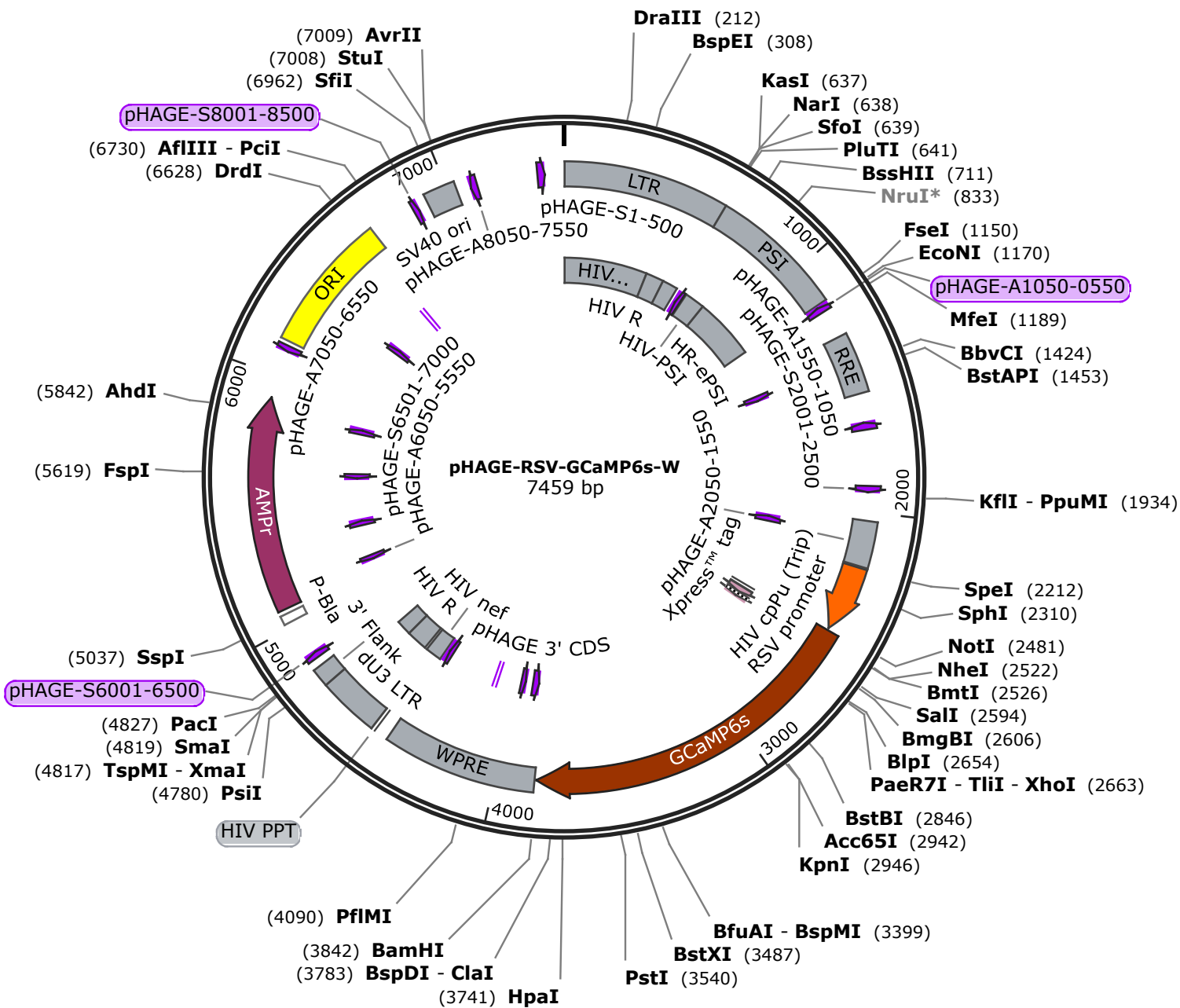


plasmid HIV Alex George Enhanced.



5' t g g a a g g g c t a a t t c a c t c c c a a a g a a g a c a a g a t a t c c t t g a t c t g t g g a t c t a
 3' a c c t t c c c g a t t a a g t g a g g g t t t c t t c t g t t c t a t a g g a a c t a g a c a c c t a g a t

55

LTR

HIV U3

c c a c a c a c a a g g c t a c t t c c c t g a t t a g c a g a a c t a c a c a c c a g g g c c a g g g g t c
 g g t g t g t g t t c c g a t g a a g g g a c t a a t c g t c t t g a t g t g t g g t c c c g g t c c c c a g

110

LTR

HIV U3

a g a t a t c c a c t g a c c t t t g g a t g g t g c t a c a a g c t a g t a c c a g t t g a g c c a g a t a
 t c t a t a g g t g a c t g g a a a c c t a c c a c g a t g t t c g a t c a t g g t c a a c t c g g t c t a t

165

LTR

HIV U3

pHAGE-A0050-8050

a g g t a g a a g a g g c c a a t a a a g g a g a g a a c a c c a g c t t g t t a c a c c c t g t g a g c c t
 t c c a t c t t c c g g t t a t t t c c t c t c t t g t g g t c g a a c a a t g t g g g a c a c t c g g a

220

LTR

HIV U3

DraIII

pHAGE-A0050-8050

g c a t g g g a t g g a t g a c c c g g a g a g a g a a g t g t t a g a g t g g a g g t t t g a c a g c c g c
 c g t a c c c t a c t a c t g g g c c t c t c t c t t c a c a a t c t c a c c t c c a a a c t g t c g g c g

275

LTR

HIV U3

c t a g c a t t t c a t c a c g t g g c c c g a g a g c t g c a t c c g g a g t a c t t c a a g a a c t g c t
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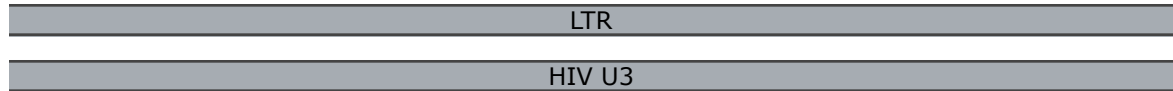
330

LTR

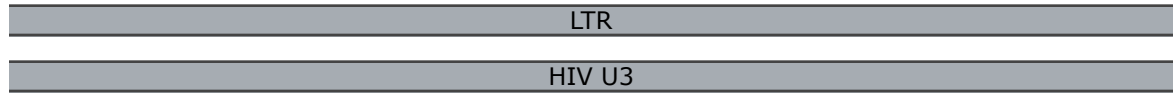
HIV U3

BspEI

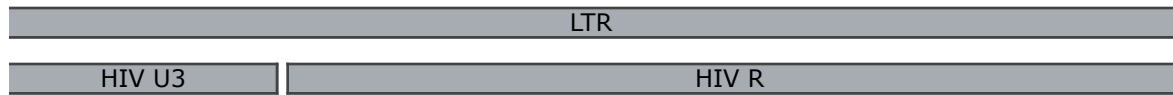
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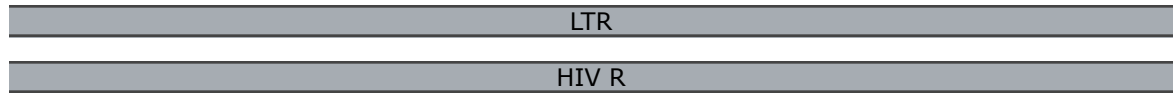
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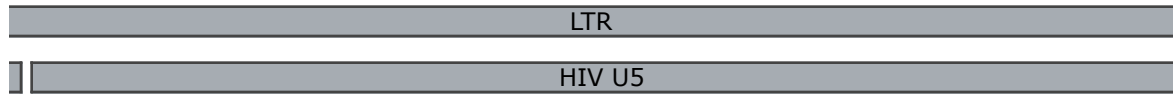
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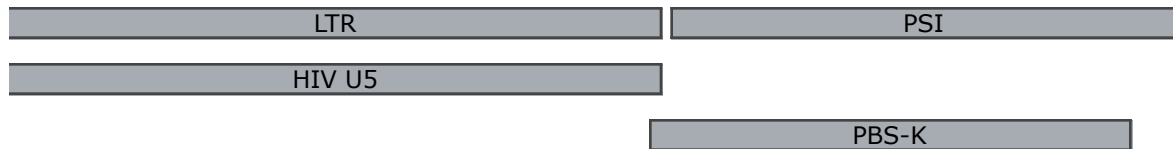
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HIV R



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BssHII

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 +-----+-----+-----+-----+-----+-----+-----+-----+
 gctttccctttggtctcctcgagagagctgcgctctgagccgaacgacttgcgcg

715

PSI

pHAGE-A0550-0050

HIV-PSI

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 +-----+-----+-----+-----+-----+-----+-----+-----+
 gtgccgttctccgctccccgccgctgaccactcatgcggtttttaaaactgatcg

770

PSI

HIV-PSI

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825

PSI

HIV-PSI

HR-ePSI

NruI*

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880

PSI

HR-ePSI

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 +-----+-----+-----+-----+-----+-----+-----+-----+
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935

PSI

HR-ePSI

pHAGE-S1001-1500

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990

PSI

HR-ePSI

cccttcagacaggatcagaagaacttagatcattatataatacagtagcaaccct
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FseI



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+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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EcoNI

MfeI



aaatataaagtagtaaaaattgaaccattaggagtagcaccaccaaggcaaga
+-----+-----+-----+-----+-----+-----+-----+-----+-----+
tttatatttcatcatttttaacttggaatcctcatcgtgggtggtccgtttct

gaagagtgggtgcagagagaaaaagagcagtgggaaataggagctttgttccttgg
+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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RRE

gttcttgggagcagcaggaagcactatggg'gcagcgtcaatgacgctgacggt
+-----+-----+-----+-----+-----+-----+-----+-----+-----+
caagaaccctcgtcgtccttcgtgatacccgctcgcagttactgagactgccat

RRE

BbvCI

caggccagacaattattgtctggtatagtgacagcagcagaacaatttgctgaggg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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1430

RRE

pHAGE-S1501-2000

BstAPI

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 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gataactccgctgtgtagacaacggttgagtgtcagaccccgtagttcgtcga

1485

RRE

ccaggcaagaatcctggctgtggaaagatacctaaaggatcaacagctcctgggg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 ggtccgttcttaggaccgacacctttctatggatttcctagttgtcgaggacccc

1540

RRE

at t t g g g g t t g c t c t g g a a a c t c a t t t g c a c c a c t g c t g t g c c t t g g a a t g c t a
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 t a a a c c c c a a c g a g a c c t t t t g a g t a a a c g t g g t g a c g a c a c g g a a c c t t a c g a t

1595

g t t g g a g t a a t a a a t c t c t g g a a c a g a t t t g g a a t c a c a c g a c c t g g a t g g a g t g
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 c a a c c t c a t t a t t t a g a g a c c t t g t c t a a a c c t t a g t g t g c t g g a c c t a c c t c a c

1650

pHAGE-A1550-1050

g g a c a g a g a a a t t a a c a a t t a c a c a a g c t t a a t a c a c t c c t t a a t t g a a g a a t c g
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 c c t g t c t c t t t a a t t g t t a a t g t g t t c g a a t t a t g t g a g g a a t t a a c t t c t t a g c

1705

pHAGE-A1550-1050

c a a a a c c a g c a a g a a a g a a t g a a c a a g a a t t a t t g g a a t t a g a t a a a t g g g c a a
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 g t t t t g g t c g t t c t t t t c t t a c t t g t t c t t a a t a a c c t t a a t c t a t t t a c c c g t t

1760

g t t t g t g g a a t t g g t t t a a c a t a a c a a a t t g g c t g t g g t a t a t a a a a t t a t t c a t
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 c a a a c a c c t t a a c c a a a t t g t a t t g t t t a a c c g a c a c c a t a t a t t t t a a t a a g t a

1815

a a t g a t a g t a g g a g g c t t g g t a g g t t t a a g a a t a g t t t t t g c t g t a c t t t c t a t a
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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1870

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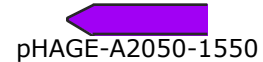
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PpuMI

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aaatggcagttatccacaatttttaaagaaaaggggggattgggggggtacag
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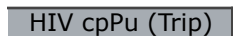


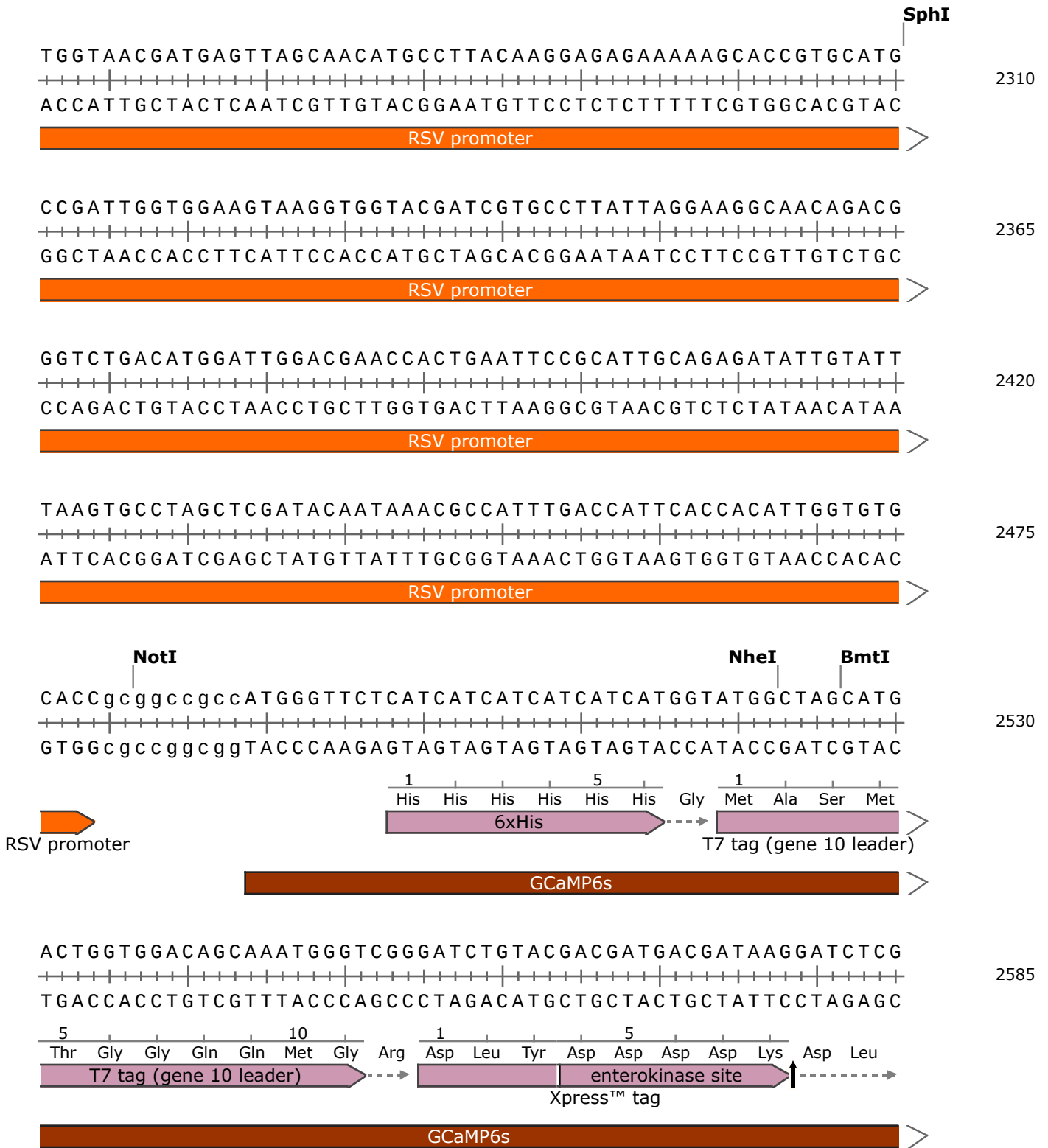
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SpeI

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GAGAAGCGCGATCACATGGTCCTGCTGGAGTTCTGTGACCGCCGCGGGGATCACTC 2915
 CTCTTCGCGCTAGTGTACCAGGACGACCTCAAGCACTGGCGGGCGGCCCTAGTGAG
 Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

TCGGCATGGACGAGCTGTACAAGGGCGGTACCGGAGGGAGCATGGTGAGCAAGGG 2970
 AGCCGTACCTGCTCGACATGTTCCCGCCATGGCCTCCCTCGTACCACTCGTTCC
 Leu Gly Met Asp Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser Lys Gly
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

CGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGCGACGTA 3025
 GCTCCTCGACAAGTGGCCCCACCACGGGTAGGACCAGCTCGACCTGCCGCTGCAT
 Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGTGAGGGCGATGCCACCTACGGCA 3080
 TTGCCGGTGTTCAAGTCGCACAGGCCGCTCCCACTCCCGCTACGGTGGATGCCGT
 Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

AGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCCAC 3135
 TCGACTGGGACTTCAAGTAGACGTGGTGGCCGTTTCGACGGGCACGGGACCGGGTG
 Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

CCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCAC 3190
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 Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
 ----- (in frame with Xpress™ tag) ----->

GCaMP6s >

ATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACATCCAGGAGC 3245
 TACTTCGTCGTGCTGAAGAAGTTCAGGCGGTACGGGCTTCCGATGTAGGTCCTCG
 Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu
 (in frame with Xpress™ tag)



GCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTT 3300
 CGTGGTAGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAA
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
 (in frame with Xpress™ tag)



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 GCTCCCGCTGTGGGACCACTTGGCGTAGCTCGACTTCCCGTAGCTGAAGTTCCTC
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 (in frame with Xpress™ tag)



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 CTGCCGTTGTAGGACCCCGTGTTCGACCTCATGTTGGACGGCCTGGTTGACTGAC
 Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Leu Pro Asp Gln Leu Thr
 (in frame with Xpress™ tag)

BfuAI
BspMI



AAGAGCAGATCGCAGAATTTAAAGAGGCTTTCTCCCTATTTGACAAGGACGGGGA 3465
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 (in frame with Xpress™ tag)



TGGGACAATAACAACCAAGGAGCTGGGGACGGTGATGCGGTCTCTGGGGCAGAAC 3520
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 Gly Thr Ile Thr Thr Lys Glu Leu Gly Thr Val Met Arg Ser Leu Gly Gln Asn
 (in frame with Xpress™ tag)



PstI

CCCACAGAAGCAGAGCTGCAGGACATGATCAATGAAGTAGATGCCGACGGTGACG
 3575
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 Pro Thr Glu Ala Glu Leu Gln Asp Met Ile Asn Glu Val Asp Ala Asp Gly Asp
 ----- (in frame with Xpress™ tag) ----->



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 3630
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 ----- (in frame with Xpress™ tag) ----->



GGACACGGAAGAAGAAATTAGAGAAGCGTTCCGGTGTGTTTGATAAGGATGGCAAT
 3685
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 ----- (in frame with Xpress™ tag) ----->



GGCTACATCAGTGCAGCAGAGCTTCGCCACGTGATGACAAACCTTGGAGAGAAGT
 3740
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 Gly Tyr Ile Ser Ala Ala Glu Leu Arg His Val Met Thr Asn Leu Gly Glu Lys
 ----- (in frame with Xpress™ tag) ----->



HpaI **ClaI**
BspDI
 TAACAGATGAAGAGGTTGATGAAATGATCAGGGAAGCAGACATCGATGGGGATGG
 3795
 ATTGTCTACTTCTCCAACTACTTTACTAGTCCCTTCGTCTGTAGCTACCCCTACC
 Leu Thr Asp Glu Glu Val Asp Glu Met Ile Arg Glu Ala Asp Ile Asp Gly Asp Gly
 ----- (in frame with Xpress™ tag) ----->



BamHI

TCAGGTAAACTACGAAGAGTTTGTACAAATGATGACAGCGAAGTGAggatcctaa
 AGTCCATTTGATGCTTCTCAAACATGTTTACTACTGTCGCTTCACTcctaggatt

3850

Gln Val Asn Tyr Glu Glu Phe Val Gln Met Met Thr Ala Lys *
 ----- (in frame with Xpress™ tag) ----->

WPRE

GCaMP6s

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3905

WPRE

pHAGE 3' CDS

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3960

WPRE

< **pHAGE 3' CDS**

pHAGE-A3550-3050

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4015

WPRE

< **pHAGE-A3550-3050**

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4070

WPRE

PfIMI

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4125

WPRE

pHAGE-S4001-4500

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 WPRE

pHAGE-S4001-4500

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 WPRE

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 WPRE

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 aagggcgccggacgacggccgagacgccgggagaaggcgcagaagcgggaagcggga
 WPRE

oligo #91 sequencing sense

cagacgagtcggatctcccttggggccgctccccgcctgagatcctttaagacc 4455
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gtctgctcagcctagagggaaccggcggaggggcggactctaggaattctgg
 WPRE HIV nef

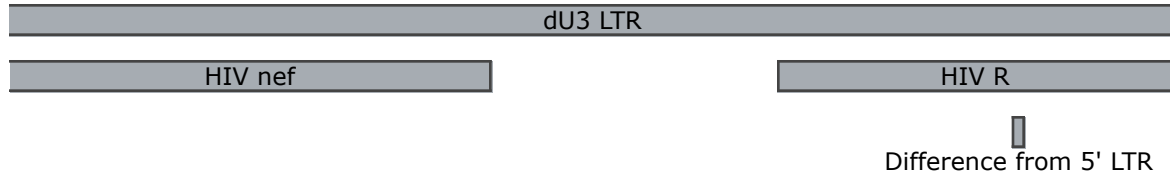
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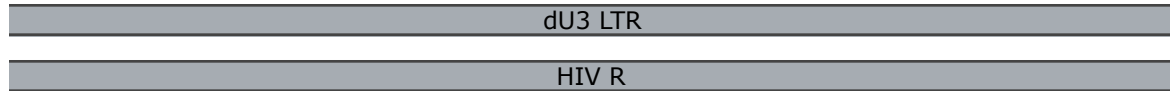
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HIV nef

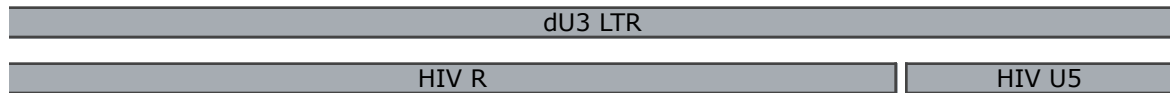
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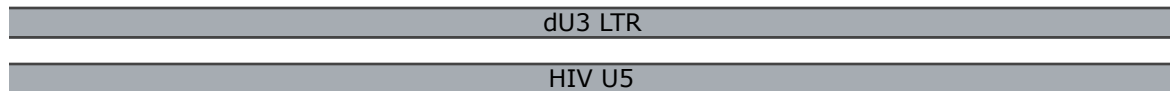
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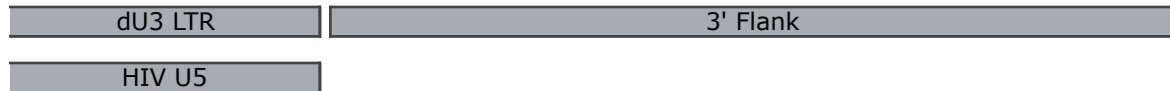
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PsiI

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XmaI
TspMI SmaI PacI

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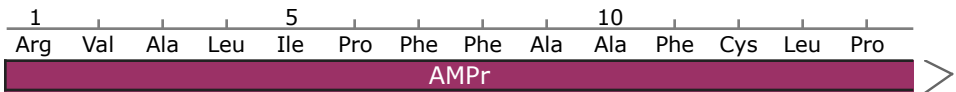
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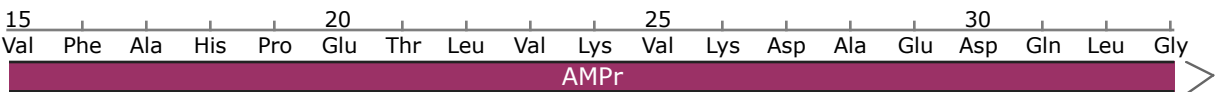
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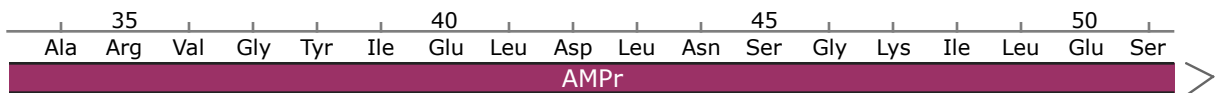
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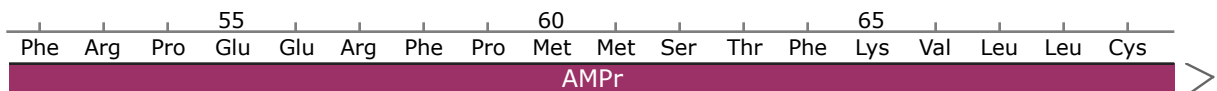
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5665

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5720

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5775

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AMPr

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5830

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AMPr

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5885

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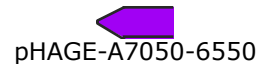
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 ORI

6490

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6545

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6600

DrdI

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6655

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 ORI

6710

PciI
AfIII

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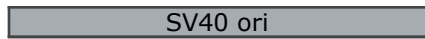
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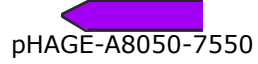
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7425

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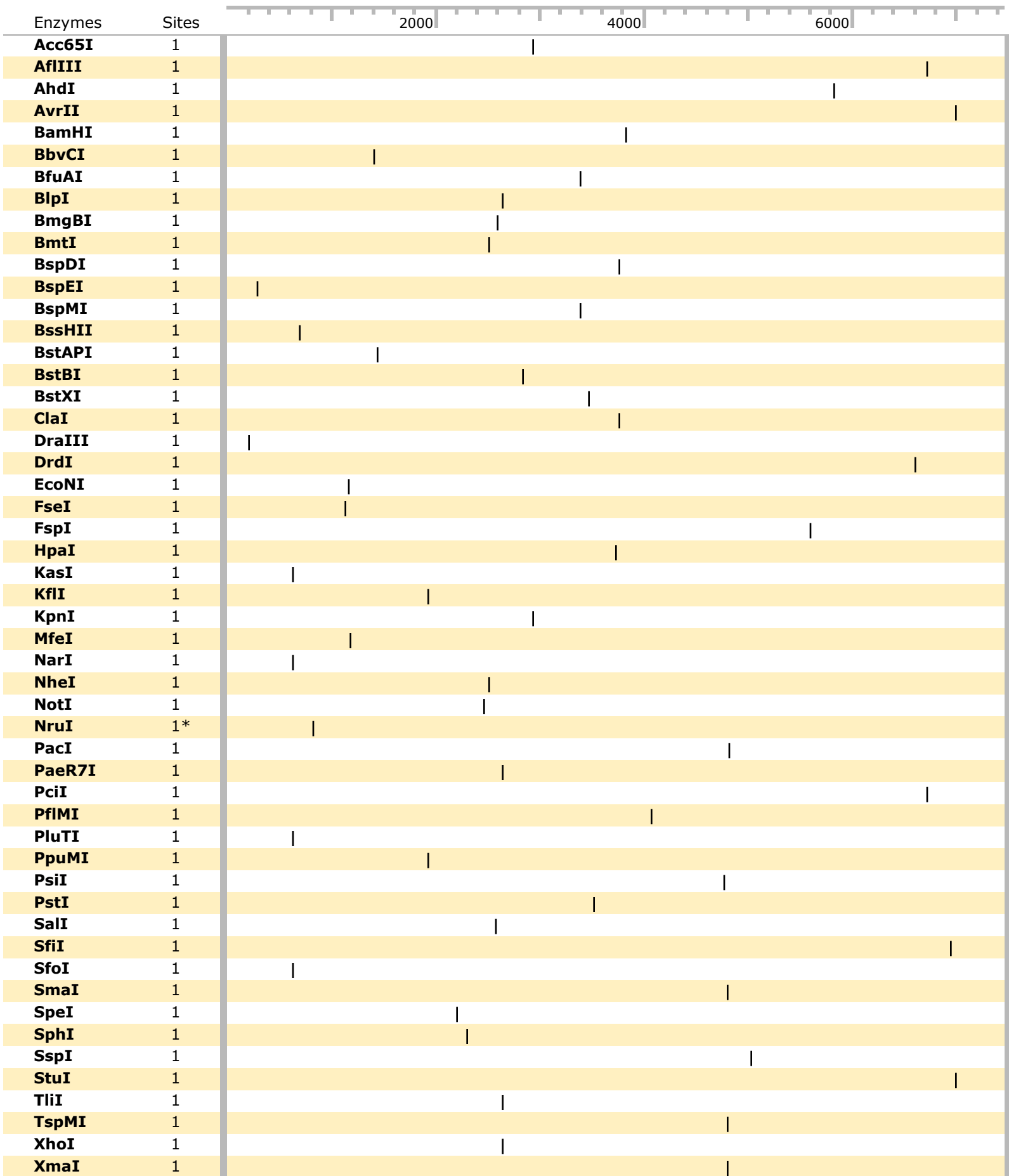
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... 7459

Enzymes	Sites	
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AflIII	1	6730
AhdI	1	5842
AvrII	1	7009
BamHI	1	3842
BbvCI	1	1424
BfuAI	1	3399
BlpI	1	2654
BmgBI	1	2606
BmtI	1	2526
BspDI	1	3783
BspEI	1	308
BspMI	1	3399
BssHII	1	711
BstAPI	1	1453
BstBI	1	2846
BstXI	1	3487
ClaI	1	3783
DraIII	1	212
DrdI	1	6628
EcoNI	1	1170
FseI	1	1150
FspI	1	5619
HpaI	1	3741
KasI	1	637
KflI	1	1934
KpnI	1	2946
MfeI	1	1189
NarI	1	638
NheI	1	2522
NotI	1	2481
NruI	1*	833*
PacI	1	4827
PaeR7I	1	2663
PciI	1	6730
PfIMI	1	4090
PluTI	1	641
PpuMI	1	1934
PsiI	1	4780
PstI	1	3540
SalI	1	2594
SfiI	1	6962
SfoI	1	639
SmaI	1	4819
SpeI	1	2212
SphI	1	2310
SspI	1	5037
StuI	1	7008
TliI	1	2663
TspMI	1	4817
XhoI	1	2663
XmaI	1	4817

Enzymes



































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





















































Enzymes





























Sites



Feature	Location	Size (bp)			Type
✓ LTR	1 .. 636	636			misc_feature
/gene	= LTR				
/note	= Long Terminal Repeat				
✓ HIV U3	1 .. 453	453			misc_feature
/note	= Full Length HIV U3				
✓ pHAGE-A0050-8050	158 .. 179	22			primer_bind
✓ pHAGE-S501-1000	401 .. 421	21			primer_bind
✓ HIV R	454 .. 551	98			misc_feature
/note	= HIV Repeat Region				
✓ HIV U5	552 .. 636	85			misc_feature
/note	= HIV U5				
✓ PBS-K	636 .. 658	23			misc_feature
/note	= tRNA binding site for Lysine tRNA				
✓ PSI	637 .. 1155	519			misc_feature
/gene	= psi				
/product	= pbs-gag				
/note	= PSI Packaging Sequence				
✓ pHAGE-A0550-0050	667 .. 690	24			primer_bind
✓ HIV-PSI	697 .. 806	110			misc_feature
✓ HR-ePSI	807 .. 1144	338			misc_feature
✓ pHAGE-S1001-1500	898 .. 919	22			primer_bind
✓ pHAGE-A1050-0550	1166 .. 1189	24			primer_bind
✓ RRE	1303 .. 1536	234			misc_feature
/gene	= rre				
/product	= minimal RRE				
/note	= Rev Responsive Element				
✓ pHAGE-S1501-2000	1391 .. 1417	27			primer_bind
✓ pHAGE-A1550-1050	1650 .. 1678	29			primer_bind

Feature	Location	Size (bp)			Type
✓ pHAGE-S2001-2500	1898 .. 1922	25			primer_bind
✓ HIV cpPu (Trip)	2034 .. 2211	178			misc_feature
/gene	= cppt				
/note	= Central Poly Purine Track				
✓ pHAGE-A2050-1550	2084 .. 2112	29			primer_bind
✓ RSV promoter	2218 .. 2479	262			promoter
/note	= Rous sarcoma virus enhancer/promoter				
✓ GCaMP6s	2489 .. 3841	1353			misc_feature
✓ 6xHis	2498 .. 2515	18			CDS
/product	= 6xHis affinity tag				
/translation	= HHHHHH				
	6 amino acids = 840.9 Da				
✓ T7 tag (gene 10 leader)	2519 .. 2551	33			CDS
/product	= leader peptide from bacteriophage T7 gene 10				
/note	= promotes efficient translation in <i>E. coli</i>				
/translation	= MASMTGGQQMG				
	11 amino acids = 1.1 kDa				
✓ Xpress™ tag	2555 .. 2578	24			CDS
▶ 2 segments					
/product	= Xpress™ epitope tag, including an enterokinase recognition and cleavage site				
/translation	= DLY,DDDDK				
	8 amino acids = 998.0 Da				
✓ WPRE	3848 .. 4439	592			misc_feature
/gene	= WPRE				
/note	= Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element				
✓ pHAGE 3' CDS	3876 .. 3910	35			primer_bind
✓ pHAGE-A3550-3050	3954 .. 3977	24			primer_bind
✓ pHAGE-S4001-4500	4107 .. 4127	21			primer_bind

Feature	Location	Size (bp)			Type
✓ oligo #91 sequencing sense	4400 .. 4423	24			primer_bind
✓ pHAGE-A4050-3550	4435 .. 4457	23			primer_bind
✓ HIV nef	4445 .. 4533	89			misc_feature
/note	= HIV nef				
✓ HIV PPT	4495 .. 4509	15			misc_feature
/note	= Polypurine Tract (Not degraded by RNaseH so it can serve as a primer for + strand DNA synthesis during life cycle)				
✓ dU3 LTR	4510 .. 4745	236			misc_feature
/gene	= dU3 LTR				
/note	= Deleted U3 Long-Terminal Repeat				
✓ HIV R	4547 .. 4662	116			misc_feature
/note	= HIV Repeat Region				
✓ Difference from 5' LTR	4558 .. 4558	1			modified_base
/note	= This T is a C in the 5' LTR				
✓ HIV U5	4663 .. 4745	83			misc_feature
/note	= HIV U5				
✓ pHAGE-S4501-5000	4743 .. 4774	32			primer_bind
✓ 3' Flank	4746 .. 4816	71			misc_feature
/note	= Chromosomal 3' Flanking Sequence carried over from original HIV integration site				
✓ pHAGE-S6001-6500	4849 .. 4871	23			primer_bind
✓ P-Bla	5020 .. 5054	35			promoter
/note	= Beta Lactamase Promoter				

Feature	Location	Size (bp)			Type
✓ AMPr	5073 .. 5912	840			CDS
/gene	= AMPr				
/product	= beta-lactamase (mature form)				
/note	= Ampicillin Resistance Gene				
/translation	= RVALIPFFAAFCLP VF AHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRPEERFPMMSTFKVLLCGAVLSRVDAGC RIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLLTTIGGPKELTAF LHNMGD HVTRLDRWEPELI DERDTTMPVAMATTLRKL LTGELLTLASRQQLIDWMEADK VAGPLLR SALPAGWFIADKSGAGERGSRGIIAALGPD VVIYTTGSQATMDERNRQIAEIGASLIKHW				
	280 amino acids = 30.8 kDa				
✓ pHAGE-A6050-5550	5122 .. 5145	24			primer_bind
✓ pHAGE-S6501-7000	5322 .. 5346	25			primer_bind
✓ pHAGE-A6550-6050	5591 .. 5614	24			primer_bind
✓ pHAGE-S7001-7500	5842 .. 5866	25			primer_bind
✓ pHAGE-A7050-6550	6102 .. 6123	22			primer_bind
✓ ORI	6141 .. 6674	534			rep_origin
/gene	= ORI				
/note	= Bacterial Origin of Replication				
✓ pHAGE-S7501-8000	6346 .. 6370	25			primer_bind
✓ pHAGE-A7550-7050	6613 .. 6634	22			primer_bind
✓ pHAGE-S8001-8500	6849 .. 6868	20			primer_bind
✓ SV40 ori	6911 .. 7034	124			misc_feature
/gene	= SV40 ori				
✓ pHAGE-A8050-7550	7089 .. 7115	27			primer_bind
✓ pHAGE-S1-500	7354 .. 7382	29			primer_bind