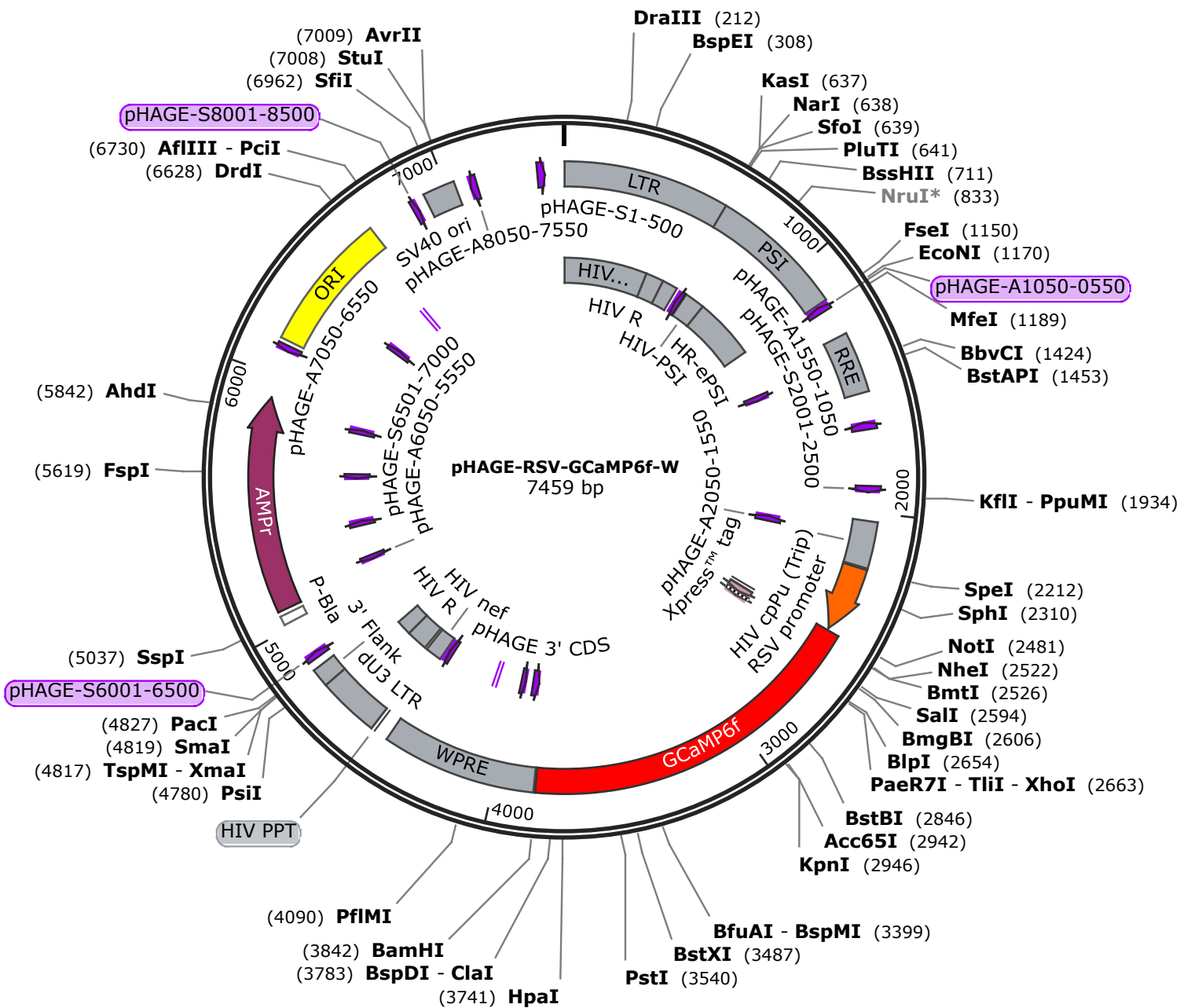
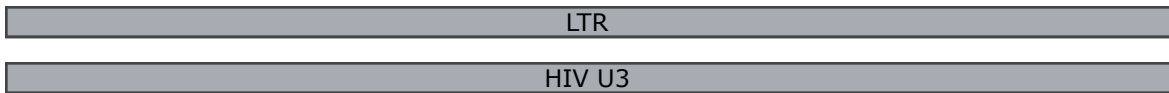


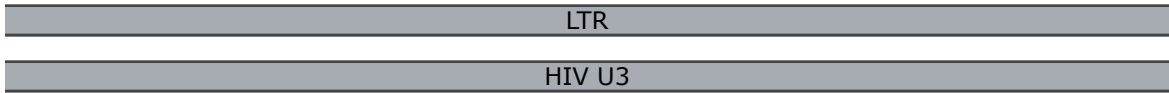
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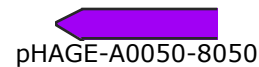
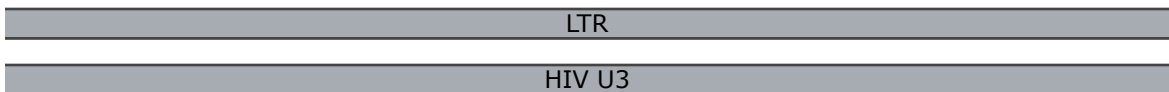
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c c a c a c a c a a g g c t a c t t c c c t g a t t a g c a g a a c t a c a c a c c a g g g c c a g g g g t c
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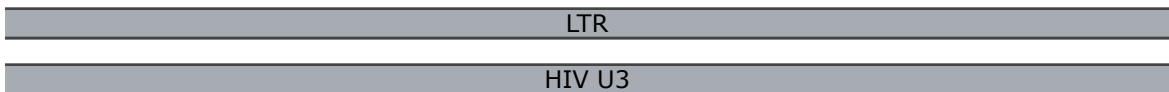


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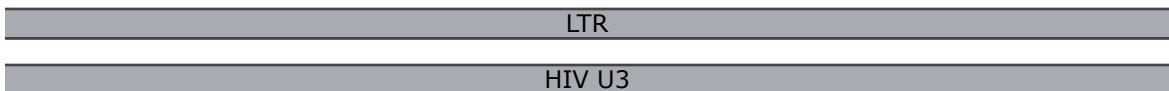


DraIII

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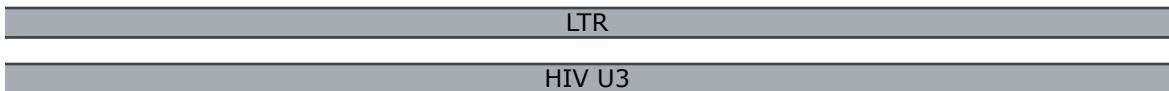


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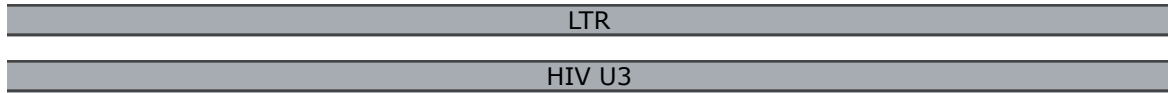
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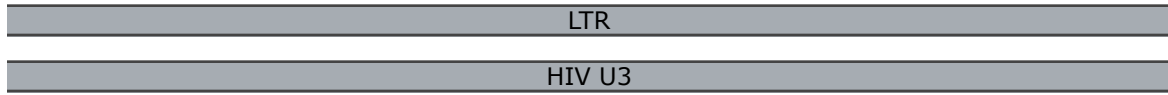
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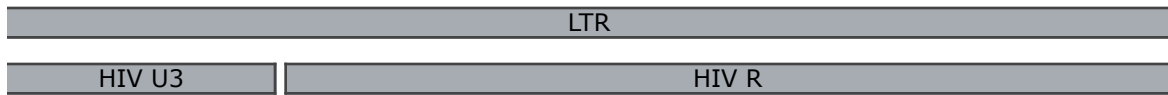
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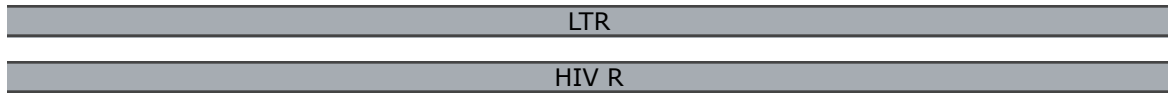
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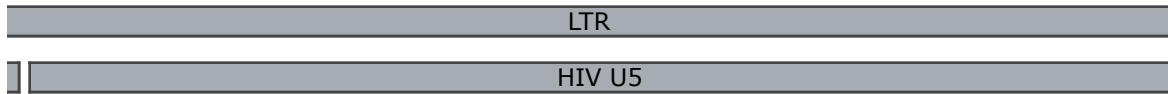
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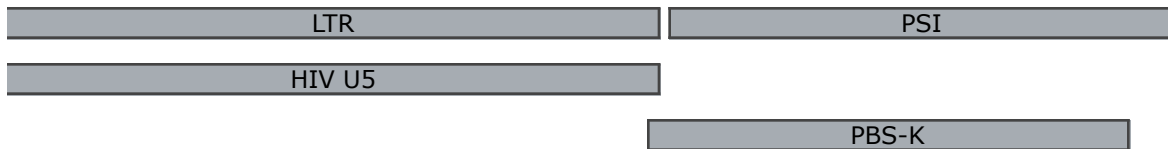


HIV R



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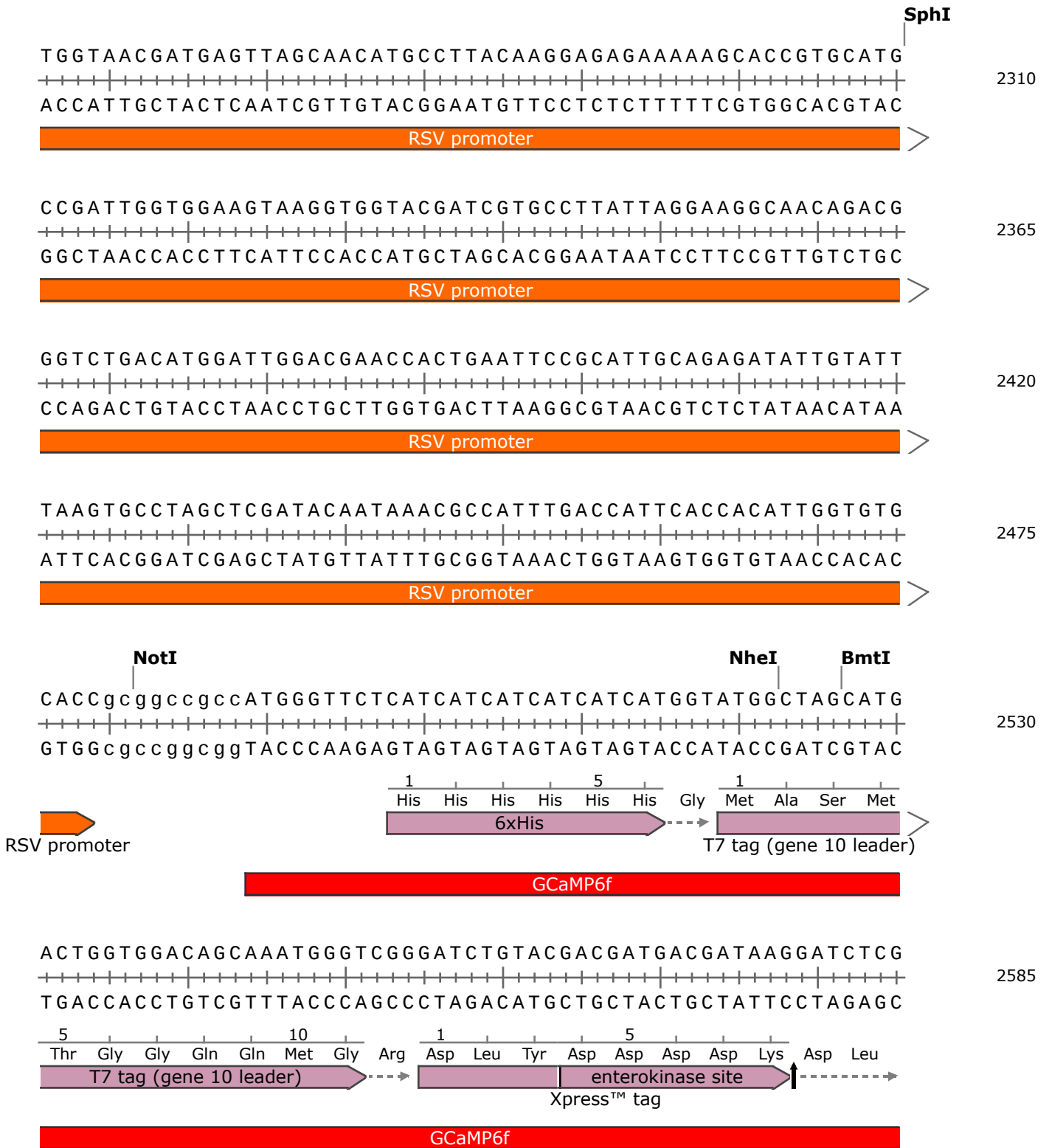
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 (in frame with Xpress™ tag)

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GCaMP6f

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 (in frame with Xpress™ tag)

GCaMP6f

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BspMI

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 (in frame with Xpress™ tag)

GCaMP6f

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GCaMP6f

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GCaMP6f

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WPRE

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WPRE

pHAGE 3' CDS

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WPRE

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HIV nef

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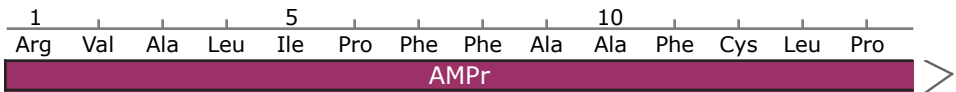
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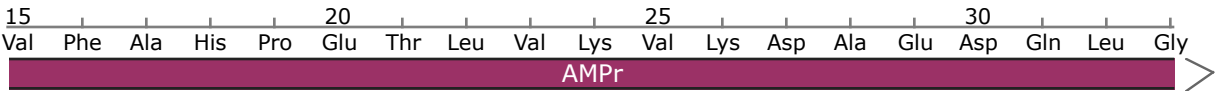
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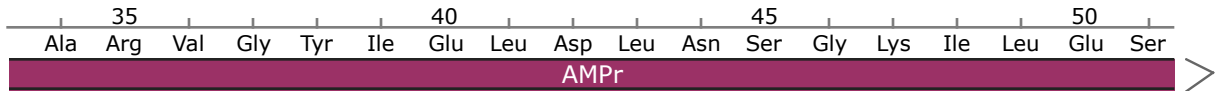
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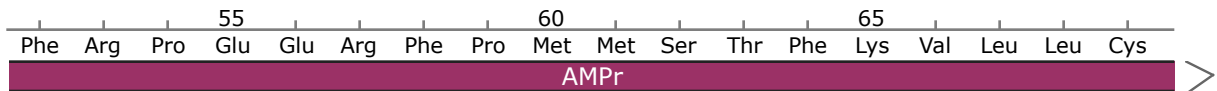
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5830

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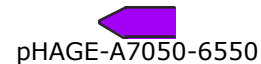
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ORI

DrdI

ctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtcagg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gacagcccaaagcgggtggagactgaactcgcagctaaaaaacactacgagcagtcc 6655

ORI

← **pHAGE-A7550-7050**

ggggcggagcctatggaaaaacgccagcaacgcggcctttttacggttcctggcc
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 cccgcctcggatacctttttgcggtcgttgcgcccggaaaaatgccaaaggaccgg 6710

ORI

PciI
AfIII

ttttgctggccttttgctcacatgttctttcctgcggttatccccctgattctgtgg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 aaaacgaccggaaaacgagtgtacaagaaaggacgcaataggggactaagacacc 6765

ataaccgtattaccgcctttgagtgagctgataccgctcgcgcagccgaacgac
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 tattggcataatggcggaaactcactcgactatggcgagcggcgtcggcttgctg 6820

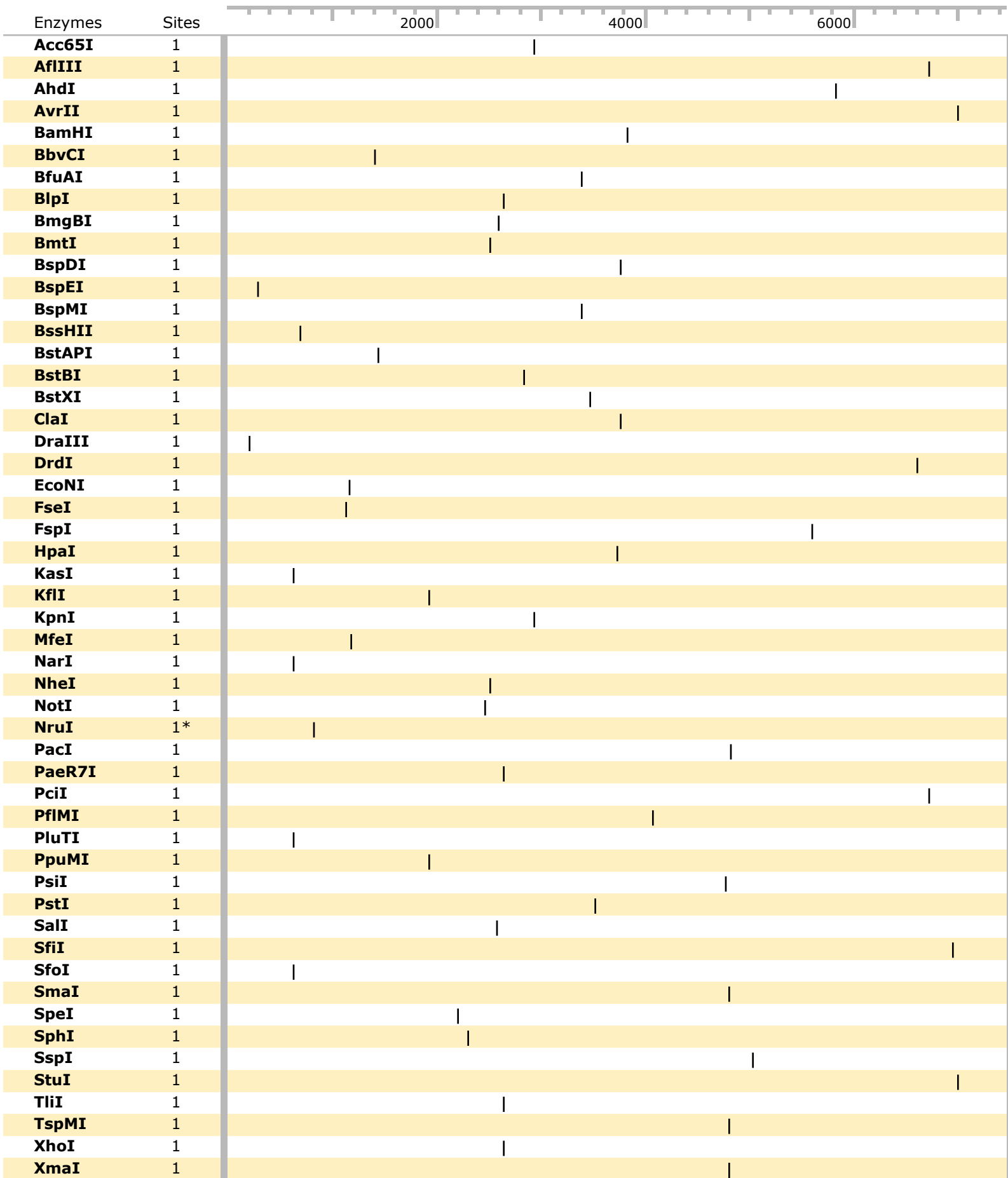
cgagcgcagcagtcagtgagcggaggaagcgggaagagcgcccaatacgcgaaaccg
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 gctcgcgtcgcctcagtcactcgtccttccgcttctcgcgggttatgcggtttggc 6875

pHAGE-S8001-8500 →

Enzymes	Sites	
Acc65I	1	2942
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



































Sites























































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				
✓ GCaMP6f	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 RIHYSQNDLVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLLTTIGGPKELT AFLHNMGDHVTRLDRWEPELI DERDTTMPVAMATTLRKLTLGELLTLASRQQLIDWMEADK 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