

gatccaccaggcccctgaagctgaaggacggggccactacacctggatggatcaag  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 ctaggtggtccgggacttcgacttcgtccgtccgtatggaccacctcaagttc  
 tdTomato > 3025

accatctacatggccaagaagccccgtgcacactgccccgtactactacgtggaca  
 +-----+-----+-----+-----+-----+-----+-----+-----+  
 tggtagatgtaccggttttcggcacgttgcacggccatgtatgtatgtgcacctgt  
 tdTomato > 3080

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 +-----+-----+-----+-----+-----+-----+-----+-----+  
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 tdTomato > 3135

gcgcgtccgagggccgcaccacctgttccctgggcattggcacccggcagcaccggc  
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 tdTomato > 3190

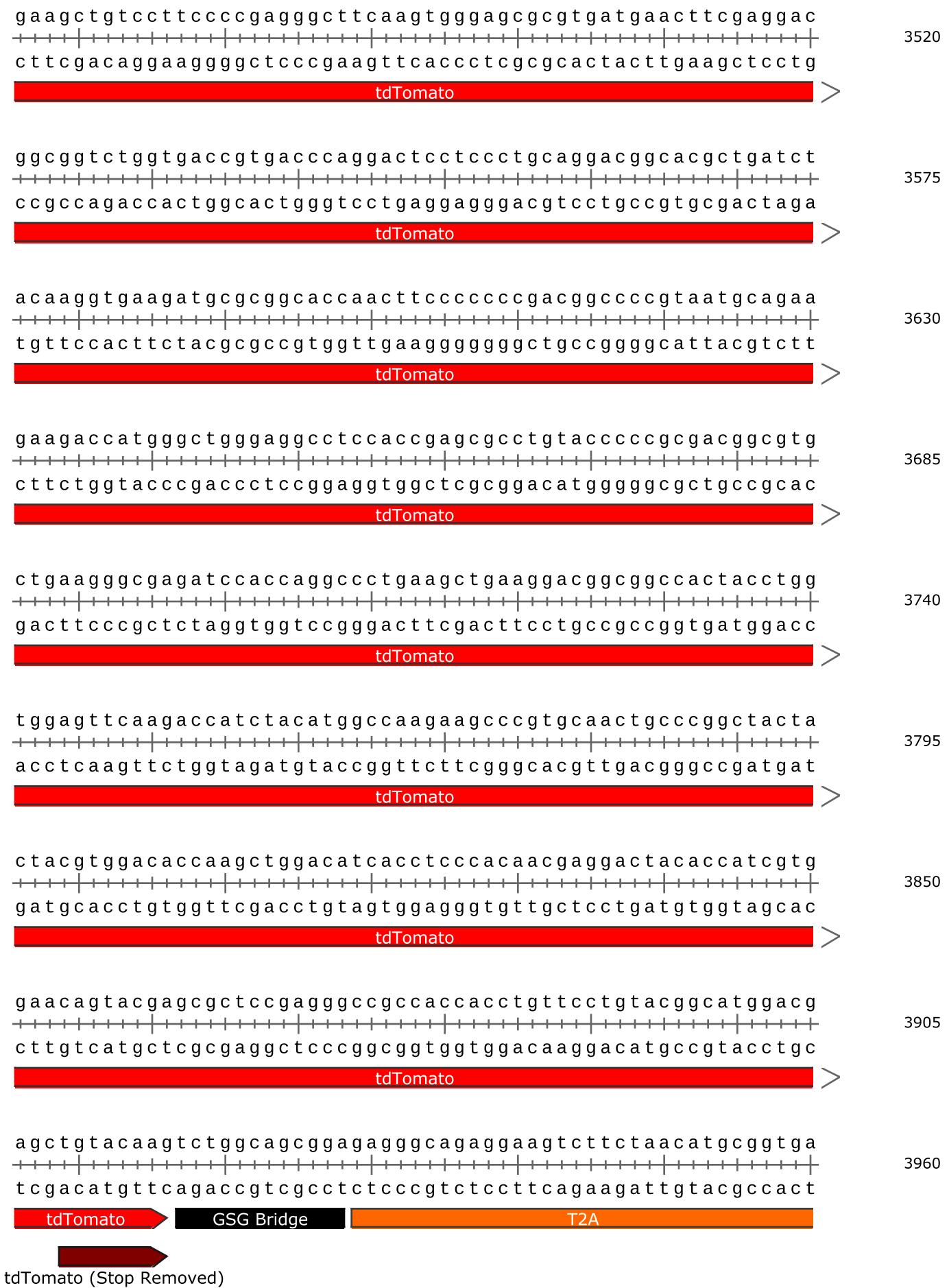
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 tdTomato > 3245

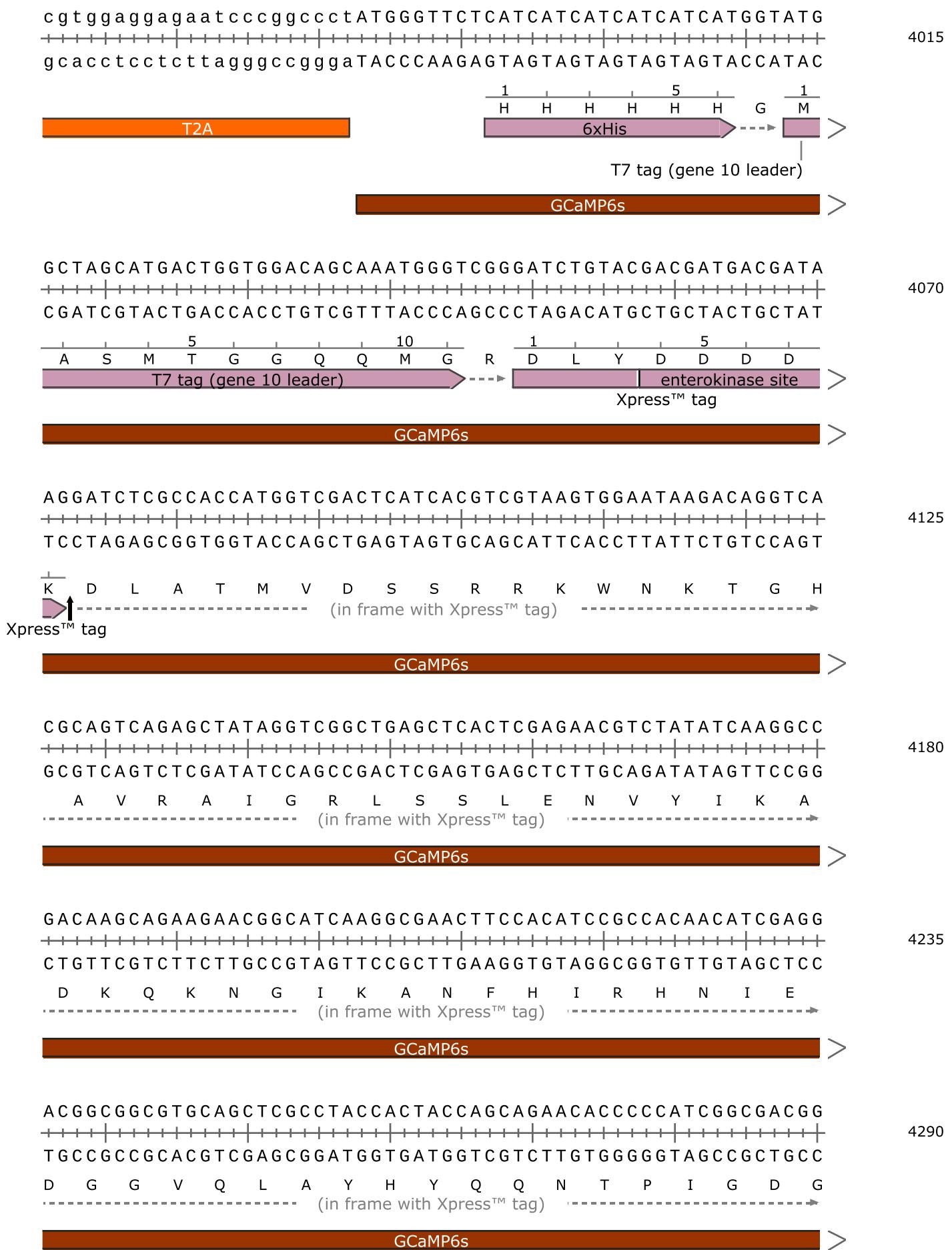
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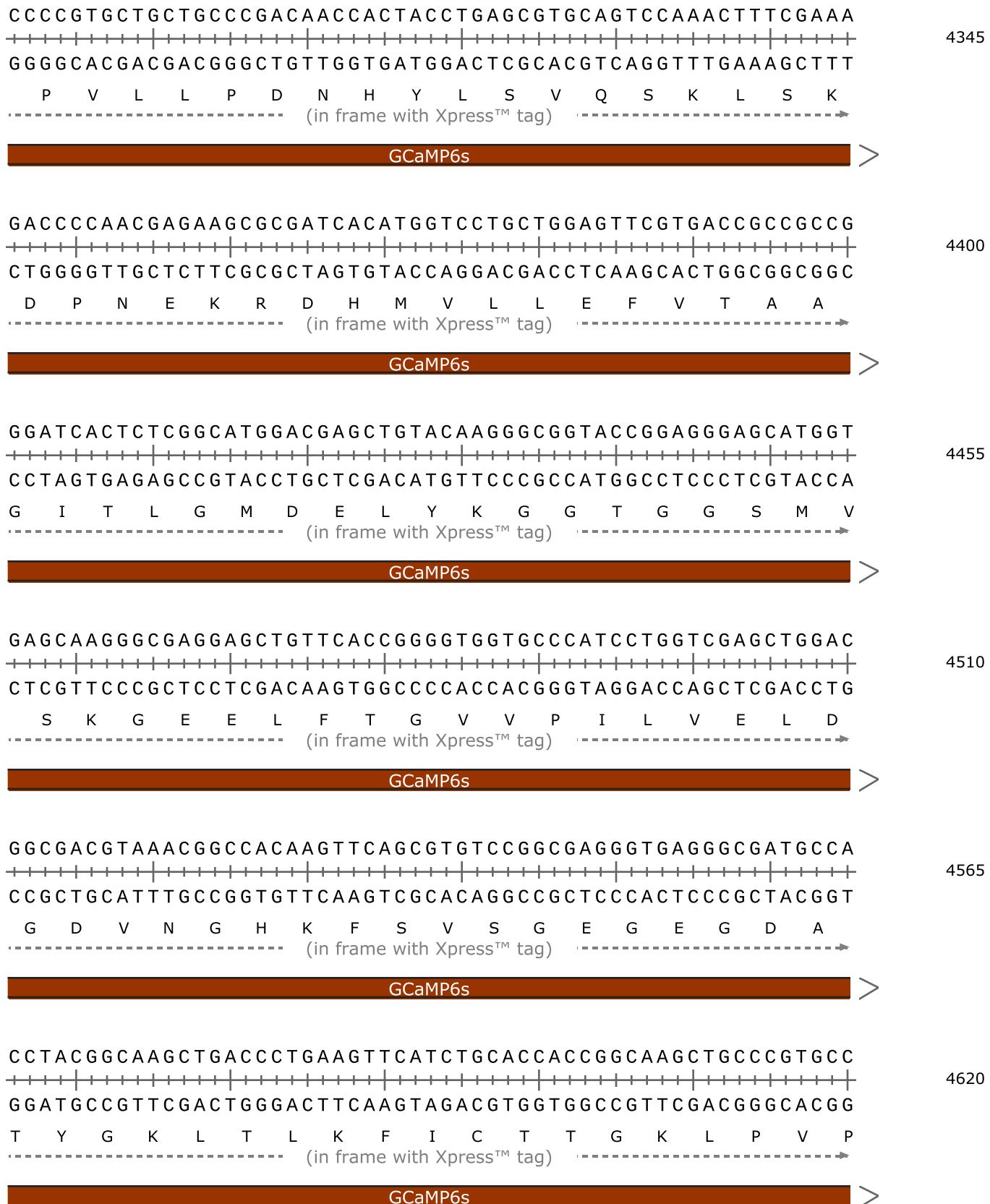
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 tdTomato > 3355

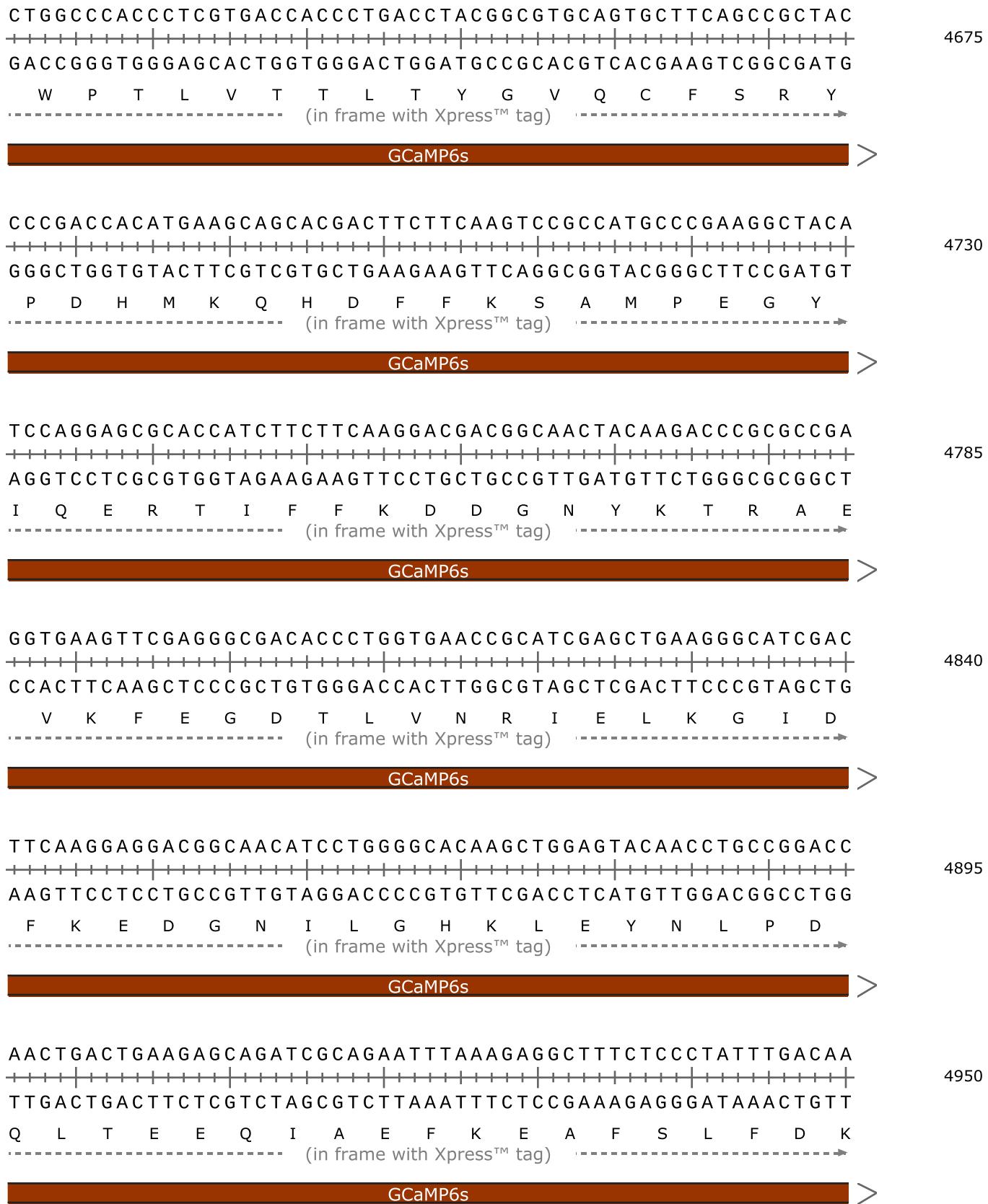
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 +-----+-----+-----+-----+-----+-----+-----+-----+  
 ttccactgggttccgcggggacggaaacggaccctgttaggacaggggggtca  
 tdTomato > 3410

tcatgtacggctcaagggtacgtgaagcacccggccacatccccattacaa  
 +-----+-----+-----+-----+-----+-----+-----+-----+  
 agtacatgccgagggtccgcattgcacttcgtggggggctgttagggctaattgtt  
 tdTomato > 3465









GGACGGGGATGGGACAATAACAACCAAGGAGCTGGGGACGGTGTGCCTCTCTG  
CCTGCCCTACCCCTGTTATTGTTGGTCTCGACCCCTGCCACTACGCCAGAGAC  
D G D G T I T T K E L G T V M R S L  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

5005

GGGCAGAACCCCACAGAACAGCAGAGCTGCAGGACATGATCAATGAAGTAGATGCCG  
CCCGTCTTGGGTGTCCTCGTCTCGACGTCTGTACTAGTTACTTCATCTACGGC  
G Q N P T E A E L Q D M I N E V D A  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

5060

ACGGTGACGGCACAAATGACTTCCCTGAGTTCTGACAATGATGGCAAGAAAAAT  
TGCCACTGCCGTGTTAGCTGAAGGGACTCAAGGACTGTTACTACC GTTCTTTTA  
D G D G T I D F P E F L T M M A R K M  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

5115

GAAATACAGGGACACGGAAAGAAGAAATTAGAGAACGCGTTGGTGTGTTGATAAG  
CTTATGTCCCTGTGCCTTCTTAAATCTCTCGCAAGCCACACAAACTATTC  
K Y R D T E E E I R E A F G V F D K  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

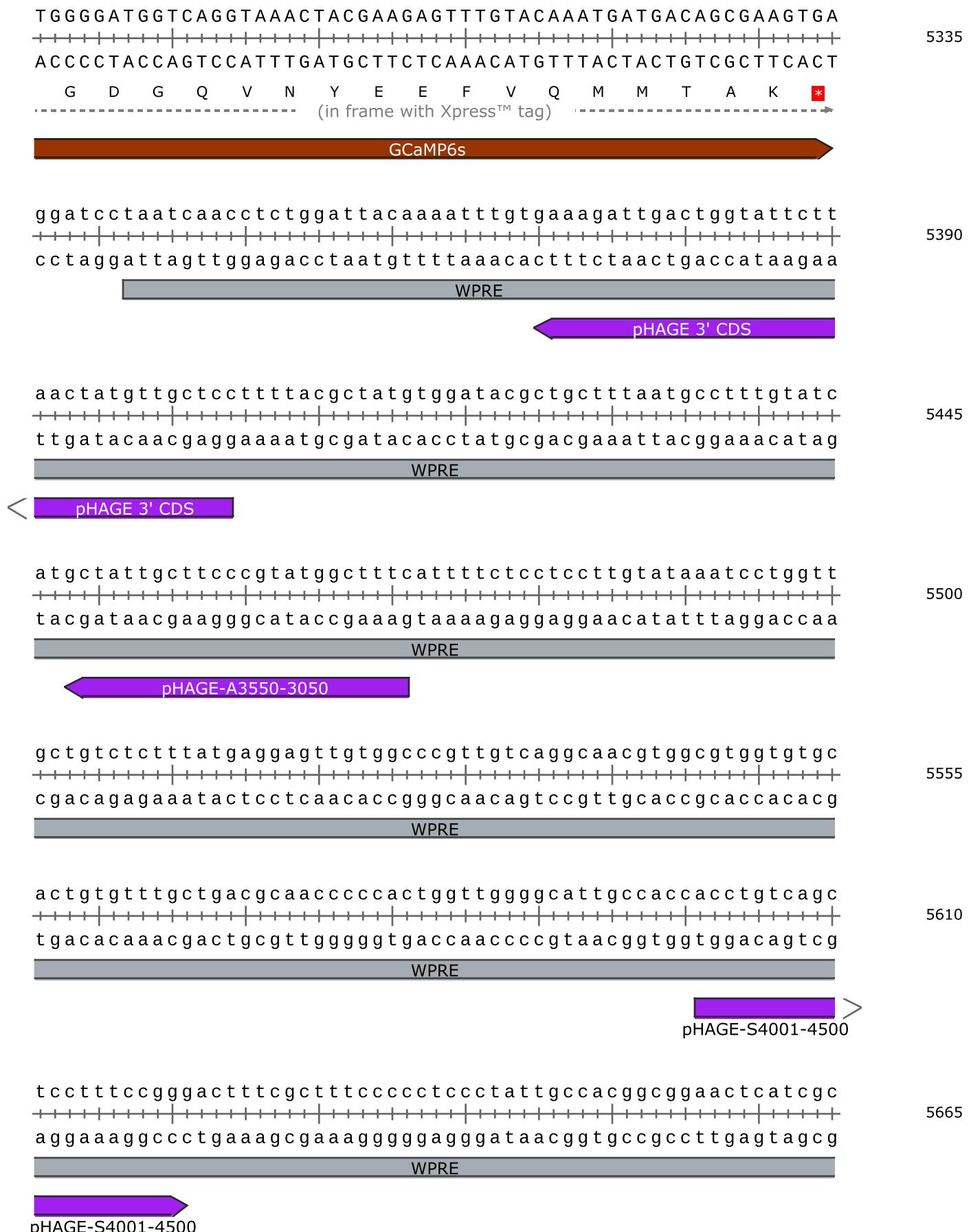
5170

GATGGCAATGGCTACATCAGTGCAGCAGAGCTTCGCCACGTGATGACAAACCTG  
CTACCGTTACCGATGTTAGTCACGTCGTCTCGAAGCGGTGCACTACTGTTGGAAC  
D G N G Y I S A A E L R H V M T N L  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

5225

GAGAGAAGTTAACAGATGAAGAGGTTGATGAAATGATCAGGGAAAGCAGACATCGA  
CTCTCTTCAATTGTTACTTCTCCAACACTTTACTAGTCCCTCGTCTGTAGCT  
G E K L T D E E V D E M I R E A D I D  
----- (in frame with Xpress™ tag) ----->  
**GCaMP6s** >

5280



The figure displays a sequence alignment of the HIV-1 genome across five distinct regions, each with a unique color-coded bar indicating its identity. The regions are:

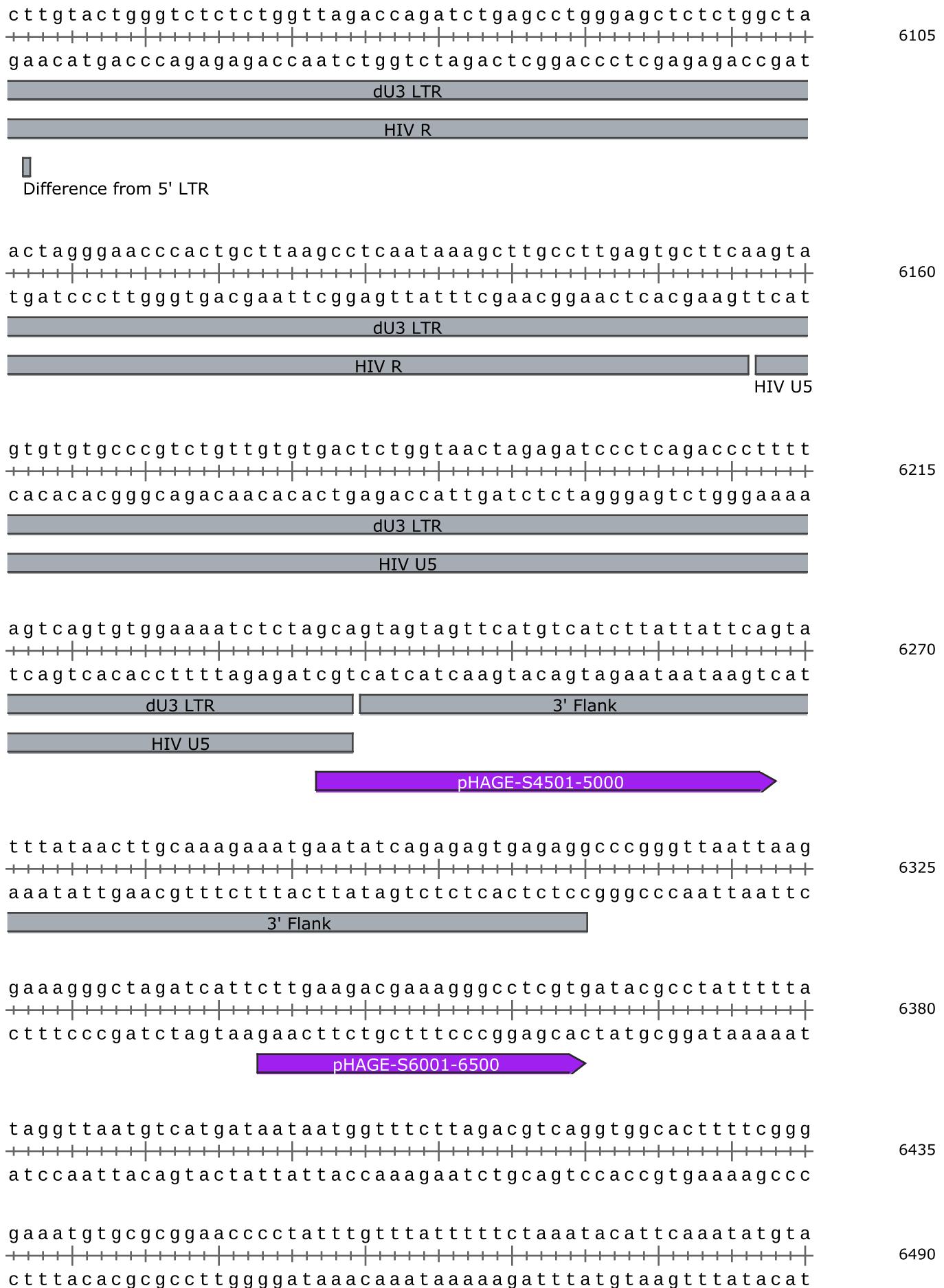
- WPRE**: White bar, located at positions 5720, 5775, and 5830.
- HIV nef**: Gray bar, located at positions 5940 and 5995.
- pHAGE-A4050-3550**: Purple bar, located at position 5940.
- HIV PPT**: Purple bar, located at position 5995.
- dU3 LTR**: Gray bar, located at position 6050.

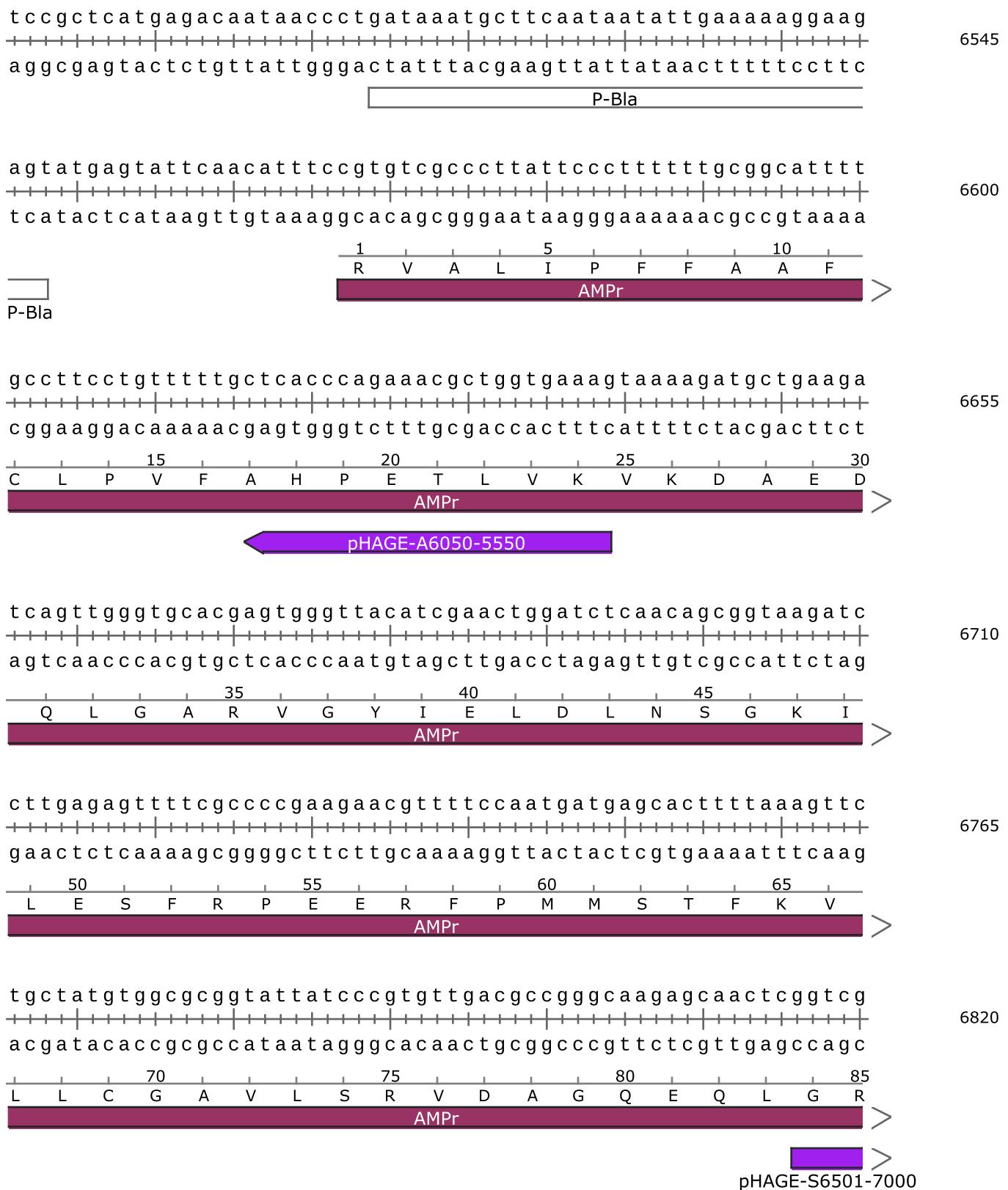
Below the sequence lines, labels indicate the sequencing sense for each primer:

- oligo #91 sequencing sense (points right)
- pHAGE-A4050-3550 (points left)
- HIV R (points right)

Sequence details for each region:

- Region 1 (5720, 5775, 5830):** Contains a WPRE motif. The sequence ends with "ggacctaagacgcccgtcaggaagacatgcaggaaagccggagtttagtgc".
- Region 2 (5940):** Contains a WPRE motif and the HIV nef gene. The sequence ends with "gaagcgggagtctgctcagccatagagggaaaccggcgagggcgactctagg".
- Region 3 (5940):** Contains the pHAGE-A4050-3550 primer and the HIV nef gene. The sequence ends with "tttaagaccaatgacttacaaggcagctgttagatcttagccacttttaaaagaa".
- Region 4 (5995):** Contains the HIV nef gene and the HIV PPT primer. The sequence ends with "aaattctggttactaatgttccgtcgacatctagaatcggtaaaaattttctt".
- Region 5 (6050):** Contains the HIV PPT primer and the dU3 LTR. The sequence ends with "aaggggggacttggaaaggcttaattcactccaaacgaagacaagatctgttttg".





6875

ccgcatacactatttcagaatgacttggttagtactcaccagtcacagaaaag  
 ggcgtatgtgataagagtcctactgaaccaactcatgagtggtcagtgtttc  
 R I H Y S Q N D L V E Y S P V T E K  
 AMPr >

pHAGE-S6501-7000 →

6930

catcttacggatggcatgacagtaagagaattatgcagtgcgtgcataaccatga  
 gtagaatgcctaccgtactgtcattctcttaatacgtcacgacggattggact  
 H L T D G M T V R E L C S A A I T M  
 AMPr >

6985

gtgataaacactgcggccaacttacttctgacaacgatcgaggaccgaaggagct  
 cactattgtgacgcgggttgaatgaagactgttgcctcctggcttcctcga  
 S D N T A A N L L T T I G G P K E L  
 AMPr >

7040

aaccgctttttgcacaacatggggatcatgttaactcgcccttgcgttggaa  
 ttggcgaaaaaacgtgttgcctacttgcgttatggttgcctcgactgtggtgctacggacatc  
 T A F L H N M G D H V T R L D R W E  
 AMPr >

7095

ccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgttag  
 ggcctcgacttacttcggtatggttgcctcgactgtggtgctacggacatc  
 P E L N E A I P N D E R D T T M P V  
 AMPr >

← pHAGE-A6550-6050

7150

caatggcaacaacgttgcgcaaactattaactggcgaactacttactctagttc  
 gttaccgttggcaacgcgttgcataattgaccgcttgcataatgagatcgaag  
 A M A T T L R K L L T G E L L T L A S  
 AMPr >

&lt; pHAGE-A6550-6050

ccggcaacaattaatagactggatggaggcgataaagtgcaggaccacttctg  
ggccgttgttaattatctgacacctccgcctattcaacgtcctggtaagac  
R Q Q L I D W M E A D K V A G P L L  
200 205 210  
AMPr >

7205

cgctcgcccttcggctggctggttattgctgataaatctggagccggtagc  
gcgagccggaaaggccgaccacaaataacgactatttagacacctcgccactcg  
R S A L P A G W F I A D K S G A G E  
215 220 225 230  
AMPr >

7260

gtgggtctcgcggtatcattgcagcaactggggccagatggtaagccctcccgat  
cacccagagcgccatagtaacgtcgtgaccccggtctaccattcggagggcata  
R G S R G I I A A L G P D G K P S R I  
235 240 245 250  
AMPr >

7315

cgtagttatctacacgacggggagtcaaggcaactatggatgaacgaaatagacag  
gcataatagatgtgctgccctcagtcgtgatcacctacttgctttatctgtc  
V V I Y T T G S Q A T M D E R N R Q  
255 260 265  
AMPr >

7370

atcgctgagatagggtgcctactgattaagcattggtaactgtcagaccaagtt  
tagcgactctatccacggagtgactaattcgtAACCTGACAGTCTGGTTCAA  
I A E I G A S L I K H W  
270 275 280  
AMPr \* >

7425

actcatatatacttttagattgattaaaacttcattttatattaaaaggatcta  
tgagtatatatgaaatctaactaaattttgaagtaaaaattaaattttcttagat

7480

ggtgaagatcctttgataatctcatgaccaaaatcccttaacgtgagtttcg  
ccacttctaggaaaaactattagagttactgggtttaggaattgcactcaaaagc

7535

ttccactgagcgtcagacccgtagaaaagatcaaaggatcttcttgagatccctt  
aagggtgactcgcagtcgggcatctttctagttcctagaagaactcttaggaa

7590

The figure displays the genome sequence of pHAGE-A7050-6550, which is 8085 bp long. The sequence is shown as a black line with vertical tick marks. Key features include:

- Left End:** A purple arrow labeled "pHAGE-A7050-6550" pointing left.
- Right End:** A yellow arrow labeled "ORI" pointing right.
- ORI Region:** A yellow bar labeled "ORI" located at the right end of the genome.
- Comparison with pHAGE-S7501-8000:** The sequence is compared with pHAGE-S7501-8000, which is 8030 bp long. The comparison shows a high degree of similarity between the two genomes.

Sequence details:

- Line 1: 7645 bp
- Line 2: 7700 bp
- Line 3: 7755 bp
- Line 4: 7810 bp
- Line 5: 7865 bp
- Line 6: 7920 bp
- Line 7: 7975 bp
- Line 8: 8030 bp
- Line 9: 8085 bp (pHAGE-A7050-6550)

tttataatgtcctgtcggtttcgccacacctgacttgagcgtcgattttgtatg  
aaatatcaggacagccaaagcggtggagactgaactcgcagctaaaaaacactac

8140

**ORI**

◀ pHAGE-A7550-7050 ▶

ctcgtcaggggggcgagccatggaaaaacgccagcaacgcggcctttacgg  
gagcagtccccccgcctcgataccctttgcggtcgttgcgcggaaaaatgcc

8195

**ORI**

ttcctggcctttgctggcctttgctcacatgttcttcgtatcccctg  
aaggaccggaaaacgaccggaaaacgagtgtaacaagaaaggacgcaataggggac

8250

attctgtggataaccgtattaccgcctttagtgagtgagctgataccgctcgccgcag  
taagacacctattggcataatggcgaaactcactcgactatggcgagcggcgtc

8305

ccgaacgaccgagcgcagcagtcagttagcgaggaagcggaaagagcggcccaata  
ggcttgctggctcgctcgctcagtcactcgctcgttgccttctcgcggttat

8360

▶ pHAGE-S8001-8500 >

cgcaaaccgcctctcccgcggttggccgattcatatgcagcaagctcatgg  
gcgttggcgagagggggcgcaaccggctaagtaattacgtcggtcgagttacc

8415

▶ SV40 ori

phAGE-S8001-8500

ctgactaattttttattatgcagaggccgaggccgcctcgccctctgagcta  
gactgattaaaaaaaaataaacgtctccggctccggagccggagactcgat

8470

▶ SV40 ori

ttccagaagttagtgaggaggcttttggaggcctaggctttgcaaaaagctcc  
aagggtttcatcactcctccgaaaaaacctccggatccgaaaacgttttcgagg

8525

▶ SV40 ori

ccgtggcacgacaggttccgactggaaagcggcagtgagcgcacgcattaa  
ggcacccgtgtccaaaggctgaccttcgcggcgtcactcgcggtcgtaat

8580

▶ SV40 ori

atgtgagttagctcactcattaggcacccaggcttacacattatgtttccggc  
+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
tacactcaatcgagtgagtaatccgtgggtccgaaatgtgaaatacgaaggccg  
 pHAGE-A8050-7550 8635

tcgatgttgtgtggaaattgtgagggataacaattcacacaggaacagctat  
+-----+-----+-----+-----+-----+-----+-----+-----+  
agcataacaacacacaccttaaacactcgccattgttaagtgttccttgcata  
8690

gacatgattacgaatttacaaaataaaggatttttcactgcattctagttgt  
+-----+-----+-----+-----+-----+-----+-----+-----+  
ctgtactaatgcttaaagtgtttattcgtaaaaaaaagtgacgtaagatcaacac  
8745

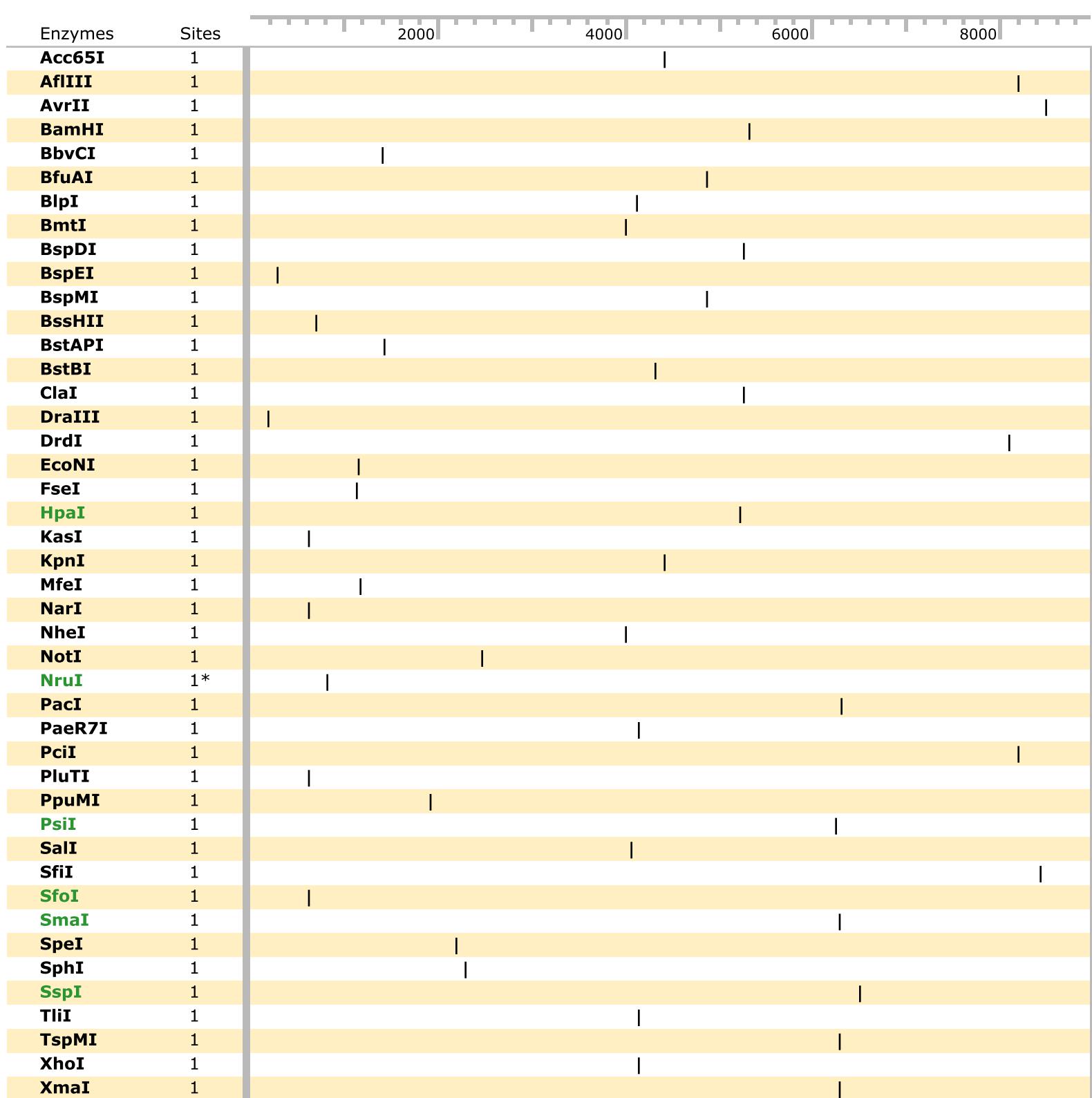
gttgtccaaactcatcaatgtatcttacatgtctggatcaactggataactca  
+-----+-----+-----+-----+-----+-----+-----+-----+  
caaacaggtttagtagttacatagaatagtagacagaccttagttgacctattgag  
8800

agctaaccaaaatcatcccaaactttccacccataccctattaccactgccaat  
+-----+-----+-----+-----+-----+-----+-----+-----+  
tcgattggtttagtaggtttaggttgaagggtggatggataatggtagcggtta  
 pHAGE-S1-500 8855

tacctgtggttcattactctaaacctgtgattccctctgaattattttcattt  
+-----+-----+-----+-----+-----+-----+-----+-----+  
atggacacccaaagtaaatgagattggacactaaggagacttaataaaagtaaaa  
 pHAGE-S1-500 8910

aaagaaattgtattgttaatatgtactacaaacttagt 3'  
+-----+-----+-----+-----+-----+-----+-----+-----+  
tttcatttaacataaacaattatacatgtttgaatcatca 5' 8953  
... 8953

Enzymes	Sites
<b>Acc65I</b>	1 4436
<b>AflIII</b>	1 8224
<b>AvrII</b>	1 8503
<b>BamHI</b>	1 5336
<b>BbvCI</b>	1 1424
<b>BfuAI</b>	1 4893
<b>BlpI</b>	1 4148
<b>BmtI</b>	1 4020
<b>BspDI</b>	1 5277
<b>BspEI</b>	1 308
<b>BspMI</b>	1 4893
<b>BssHII</b>	1 711
<b>BstAPI</b>	1 1453
<b>BstBI</b>	1 4340
<b>ClaI</b>	1 5277
<b>DraIII</b>	1 212
<b>DrdI</b>	1 8122
<b>EcoNI</b>	1 1170
<b>FseI</b>	1 1150
<b>HpaI</b>	1 5235
<b>KasI</b>	1 637
<b>KpnI</b>	1 4440
<b>MfeI</b>	1 1189
<b>NarI</b>	1 638
<b>NheI</b>	1 4016
<b>NotI</b>	1 2481
<b>NruI</b>	1* 833*
<b>PacI</b>	1 6321
<b>PaeR7I</b>	1 4157
<b>PciI</b>	1 8224
<b>PluTI</b>	1 641
<b>PpuMI</b>	1 1934
<b>PsiI</b>	1 6274
<b>SalI</b>	1 4088
<b>SfiI</b>	1 8456
<b>SfoI</b>	1 639
<b>SmaI</b>	1 6313
<b>SpeI</b>	1 2212
<b>SphI</b>	1 2310
<b>SspI</b>	1 6531
<b>TliI</b>	1 4157
<b>TspMI</b>	1 6311
<b>XhoI</b>	1 4157
<b>XmaI</b>	1 6311



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
✓ pHAGE-S2001-2500		1898 .. 1922	25			primer_bind

Feature	Location	Size (bp)			Type
✓ <b>HIV cpPu (Trip)</b>	2034 .. 2211	178			misc_feature
/gene	= cppt				
/note	= Central Poly Purine Track				
✓ <b>pHAGE-A2050-1550</b>	2084 .. 2112	29			primer_bind
✓ <b>RSV promoter</b>	2218 .. 2479	262			promoter
/note	= Rous sarcoma virus enhancer/promoter				
✓ <b>tdTomato</b>	2489 .. 3916	1428			misc_feature
/label	= tomato				
✓ <b>tdTomato (Stop Removed)</b>	3909 .. 3916	8			misc_feature
<b>Pair 1</b>	3917 .. 3982	66			misc_feature
<b>Pair 2</b>	3917 .. 3982	66			misc_feature
<b>Pair 02</b>	3917 .. 3982	66			misc_feature
<b>Pair 03</b>	3917 .. 3982	66			misc_feature
<b>Pair 05</b>	3917 .. 3982	66			misc_feature
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				

Feature	Location	Size (bp)			Type
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Reading Frame</b>	3917 .. 3982	66			misc_feature
/note	= Reading Frame				
<b>Seq #64</b>	3917 .. 3982	66			misc_feature
/note	= Seq #64				
<b>Seq eGFP_F</b>	3917 .. 3982	66			misc_feature
/note	= Seq eGFP_F				
✓ <b>GSG Bridge</b>	3917 .. 3928	12			misc_feature
✓ <b>T2A</b>	3929 .. 3982	54			misc_feature
✓ <b>GCaMP6s</b>	3983 .. 5335	1353			misc_feature
✓ <b>6xHis</b>	3992 .. 4009	18			CDS
/product	= 6xHis affinity tag				
/translation	= HHHHHH				
	6 amino acids = 840.9 Da				
✓ <b>T7 tag (gene 10 leader)</b>	4013 .. 4045	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				
✓ <b>Xpress™ tag</b>	4049 .. 4072	24			CDS
► 2 segments					
/product	= Xpress™ epitope tag, including an enterokinase recognition and cleavage site				
/translation	= DLY,DDDDK				
	8 amino acids = 998.0 Da				
✓ <b>WPRE</b>	5342 .. 5933	592			misc_feature
/gene	= WPRE				
/note	= Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element				
✓ <b>pHAGE 3' CDS</b>	5370 .. 5404	35			primer_bind

Feature	Location	Size (bp)			Type
✓ <b>pHAGE-A3550-3050</b>	5448 .. 5471	24			primer_bind
✓ <b>pHAGE-S4001-4500</b>	5601 .. 5621	21			primer_bind
✓ <b>oligo #91 sequencing sense</b>	5894 .. 5917	24			primer_bind
✓ <b>pHAGE-A4050-3550</b>	5929 .. 5951	23			primer_bind
✓ <b>HIV nef</b>	5939 .. 6027	89			misc_feature
/note	= HIV nef				
✓ <b>HIV PPT</b>	5989 .. 6003	15			misc_feature
/note	= Polypurine Tract (Not degraded by RNaseH so it can serve as a primer for + strand DNA synthesis during viral life cycle)				
✓ <b>dU3 LTR</b>	6004 .. 6239	236			misc_feature
/gene	= dU3 LTR				
/note	= Deleted U3 Long-Terminal Repeat				
✓ <b>HIV R</b>	6041 .. 6156	116			misc_feature
/note	= HIV Repeat Region				
✓ <b>Difference from 5' LTR</b>	6052 .. 6052	1			modified_base
/note	= This T is a C in the 5' LTR				
✓ <b>HIV U5</b>	6157 .. 6239	83			misc_feature
/note	= HIV U5				
✓ <b>pHAGE-S4501-5000</b>	6237 .. 6268	32			primer_bind
✓ <b>3' Flank</b>	6240 .. 6310	71			misc_feature
/note	= Chromosomal 3' Flanking Sequence carried over from original HIV integration site				
✓ <b>pHAGE-S6001-6500</b>	6343 .. 6365	23			primer_bind
✓ <b>P-Bla</b>	6514 .. 6548	35			promoter
/note	= Beta Lactamase Promoter				

Feature	Location	Size (bp)			Type
✓ <b>AMPr</b>	6567 .. 7406	840			CDS
/gene	= AMPr				
/product	= beta-lactamase (mature form)				
/note	= Ampicillin Resistance Gene				
/translation	= RVALIPFFAAFCLPVFAHPETLVVKVDAEDQLGARVGYIELDLNSGKILESRPPEERFPMMSTFKVLLCGAVLSRVDACT RRIHYSQNDLVEYSPVTEKHLDGMTVRELCASAITMSDNTAANLLLTTIGGPKELTAFLHNMGDHVTRLDRWEPEL DERDTTVPAMATTLRKLLTGELLTLASRQQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIALGPI IVVIYTTGSQATMDERNRQIAEIGASLIKHW				
	280 amino acids = 30.8 kDa				
✓ <b>pHAGE-A6050-5550</b>	6616 .. 6639	24			primer_bind
✓ <b>pHAGE-S6501-7000</b>	6816 .. 6840	25			primer_bind
✓ <b>pHAGE-A6550-6050</b>	7085 .. 7108	24			primer_bind
✓ <b>pHAGE-S7001-7500</b>	7336 .. 7360	25			primer_bind
✓ <b>pHAGE-A7050-6550</b>	7596 .. 7617	22			primer_bind
✓ <b>ORI</b>	7635 .. 8168	534			rep_origin
/gene	= ORI				
/note	= Bacterial Origin of Replication				
✓ <b>pHAGE-S7501-8000</b>	7840 .. 7864	25			primer_bind
✓ <b>pHAGE-A7550-7050</b>	8107 .. 8128	22			primer_bind
✓ <b>pHAGE-S8001-8500</b>	8343 .. 8362	20			primer_bind
✓ <b>SV40 ori</b>	8405 .. 8528	124			misc_feature
/gene	= SV40 ori				
✓ <b>pHAGE-A8050-7550</b>	8583 .. 8609	27			primer_bind
✓ <b>pHAGE-S1-500</b>	8848 .. 8876	29			primer_bind