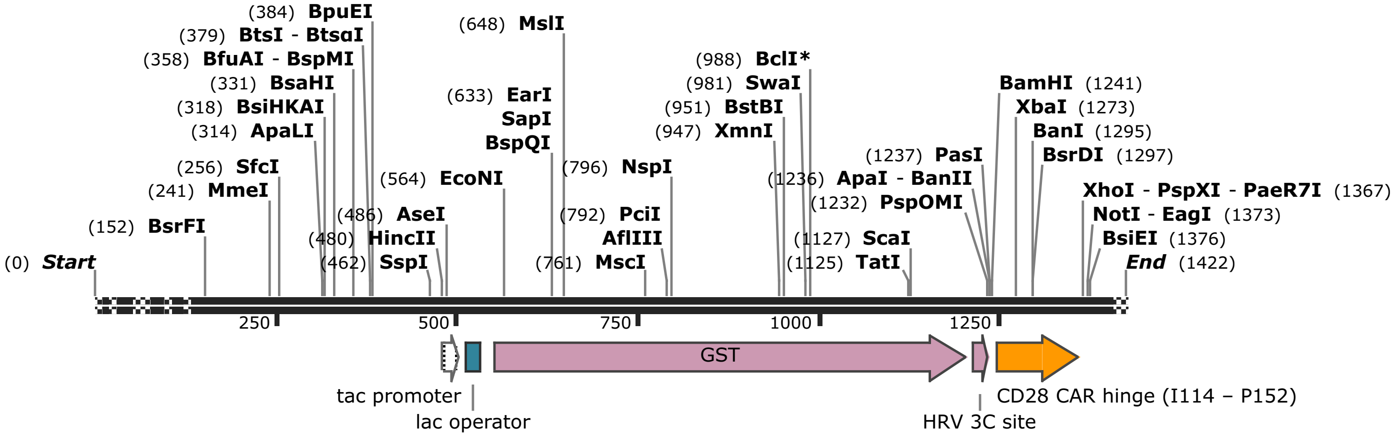
Sequence: st1_VF-1_pGEX3.dna (Linear / 1422 bp)
Enzymes: Unique 6+ Cutters (43 of 678 total)
Features: 5 total
Primers: 0 total

Unique Cutters **Bold**



st1_VF-1_pGEX3
1422 bp

```
5′ GGNNNNNNTNTNNNGNNNCCNCCNNNNNNGGGNNAANNAGNNNCCCCCNNNCCTC   55
3′ CCNNNNNNANANNNCNNNGGNGGNNNNNNCCCNNTTNNTCNNNGGGGGNNNGGAG

   NNNNNNNCCCCNNNNNNNNAGNNTTNTTTTTCCCCCCAANNGGCNNNNAGGGGGN  110
   NNNNNNNGGGGNNNNNNNNTCNNAANAAAAAGGGGGGTTNNCCGNNNNTCCCCCN

   NNGNNTTTNTNNGCGNNNNNNTTTGTCCCTCAAATGGCAANGCCGGTTAGATGGC  165
   NNCNNAAANANNCGCNNNNNNAAACAGGGAGTTTACCGTTNCGGCCAATCTACCG

   CCTTTACCCAANNNACTTTCCCATAGGTCATCGCCGTTGTTCCCCGGGAATCCGA  220
   GGAAATGGGTTNNNTGAAAGGGTATCCAGTAGCGGCAACAAGGGGCCCTTAGGCT

   CGGGTGTTNNTNNTCCCTTTAANGTGATGAAGCTGCTACAGAAGGCCANACGCGA  275
   GCCCACAANNANNAGGGAAATTNCACTACTTCGACGATGTCTTCCGGTNTGCGCT

   TTATTTTGATGGCGTGGAATTAGCTTTTCGACTGCACGGTGCACCAATGCTTCTG  330
   AATAAAACTACCGCACCTTAATCGAAAAGCTGACGTGCCACGTGGTTACGAAGAC

   GCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGC  385
   CGCAGTCCGTCGGTAGCCTTCGACACCATACCGACACGTCCAGCATTTAGTGACG

   ATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGA  440
   TATTAAGCACAGCGAGTTCCGCGTGAGGGCAAGACCTATTACAAAAAACGCGGCT

   CATCATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCG  495
   GTAGTATTGCCAAGACCGTTTATAAGACTTTACTCGACAACTGTTAATTAGTAGC

   GCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGTA  550
   CGAGCATATTACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTTTGTCAT
```

```
                EcoNI
TTCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTC      605
AAGTACAGGGGATATGATCCAATAACCTTTTAATTCCCGGAACACGTTGGGTGAG
  1     5    10    15
  M S P I L G Y W K I K G L V Q P T
GST
```

```
EarI
SapI
BspQI                                    MslI
GACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGA      660
CTGAAGAAAACCTTATAGAACTTCTTTTTATACTTCTCGTAAACATACTCGCGCT
  20    25    30    35
R L L L E Y L E E K Y E E H L Y E R D
GST
```

```
TGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAAT      715
ACTTCCACTATTTACCGCTTTGTTTTTCAAACTTAACCCAAACCTCAAAGGGTTA
   40    45    50
 E G D K W R N K K F E L G L E F P N
GST
```

```
                                            MscI
CTTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATAC      770
GAAGGAATAATATAACTACCACTACAATTTAATTGTGTCAGATACCGGTAGTATG
55    60    65    70
 L P Y Y I D G D V K L T Q S M A I I
GST
```

```
               PciI
               AflIII  NspI
GTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGA      825
CAATATATCGACTGTTCGTGTTGTACAACCCACCAACAGGTTTTCTCGCACGTCT
  75    80    85    90
R Y I A D K H N M L G G C P K E R A E
GST
```

```
GATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATT      880
CTAAAGTTACGAACTTCCTCGCCAAAACCTATAATCTATGCCACAAAGCTCTTAA
   95    100    105
 I S M L E G A V L D I R Y G V S R I
GST
```

```
GCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTG   935
CGTATATCATTTCTGAAACTTTGAGAGTTTCAACTAAAAGAATCGTTCGATGGAC
110              115            120             125
A  Y  S  K  D  F  E  T  L  K  V  D  F  L  S  K  L  P
GST

          XmnI    BstBI                          SwaI     BclI*
AAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGA   990
TTTACGACTTTTACAAGCTTCTAGCAAATACAGTATTTTGTATAAATTTACCACT
      130             135             140              145
E  M  L  K  M  F  E  D  R  L  C  H  K  T  Y  L  N  G  D
GST

TCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATAC   1045
AGTACATTGGGTAGGACTGAAGTACAACATACTGCGAGAACTACAACAAAATATG
         150            155             160
 H  V  T  H  P  D  F  M  L  Y  D  A  L  D  V  V  L  Y
GST

ATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTA   1100
TACCTGGGTTACACGGACCTACGCAAGGGTTTTAATCAAACAAAATTTTTTGCAT
165              170            175             180
M  D  P  M  C  L  D  A  F  P  K  L  V  C  F  K  K  R
GST

                   TatI ScaI
TTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATG   1155
AACTTCGATAGGGTGTTTAACTATTCATGAACTTTAGGTCGTTCATATATCGTAC
      185             190             195              200
I  E  A  I  P  Q  I  D  K  Y  L  K  S  S  K  Y  I  A  W
GST

GCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCG   1210
CGGAAACGTCCCGACCGTTCGGTGCAAACCACCACCGCTGGTAGGAGGTTTTAGC
         205            210             215
 P  L  Q  G  W  Q  A  T  F  G  G  G  D  H  P  P  K  S
GST

                    ApaI   PasI
          PspOMI    BanII          BamHI
GATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCATAGAAGTAATGTACCCCC   1265
CTAGACCTTCAAGACAAGGTCCCCGGGGACCCTAGGTATCTTCATTACATGGGGG
    1           5                  1           5
D  L  E  V  L  F  Q  G  P  L  G  S  I  E  V  M  Y  P
                                   CD28 CAR hinge (I114 – P152)
HRV 3C site
```

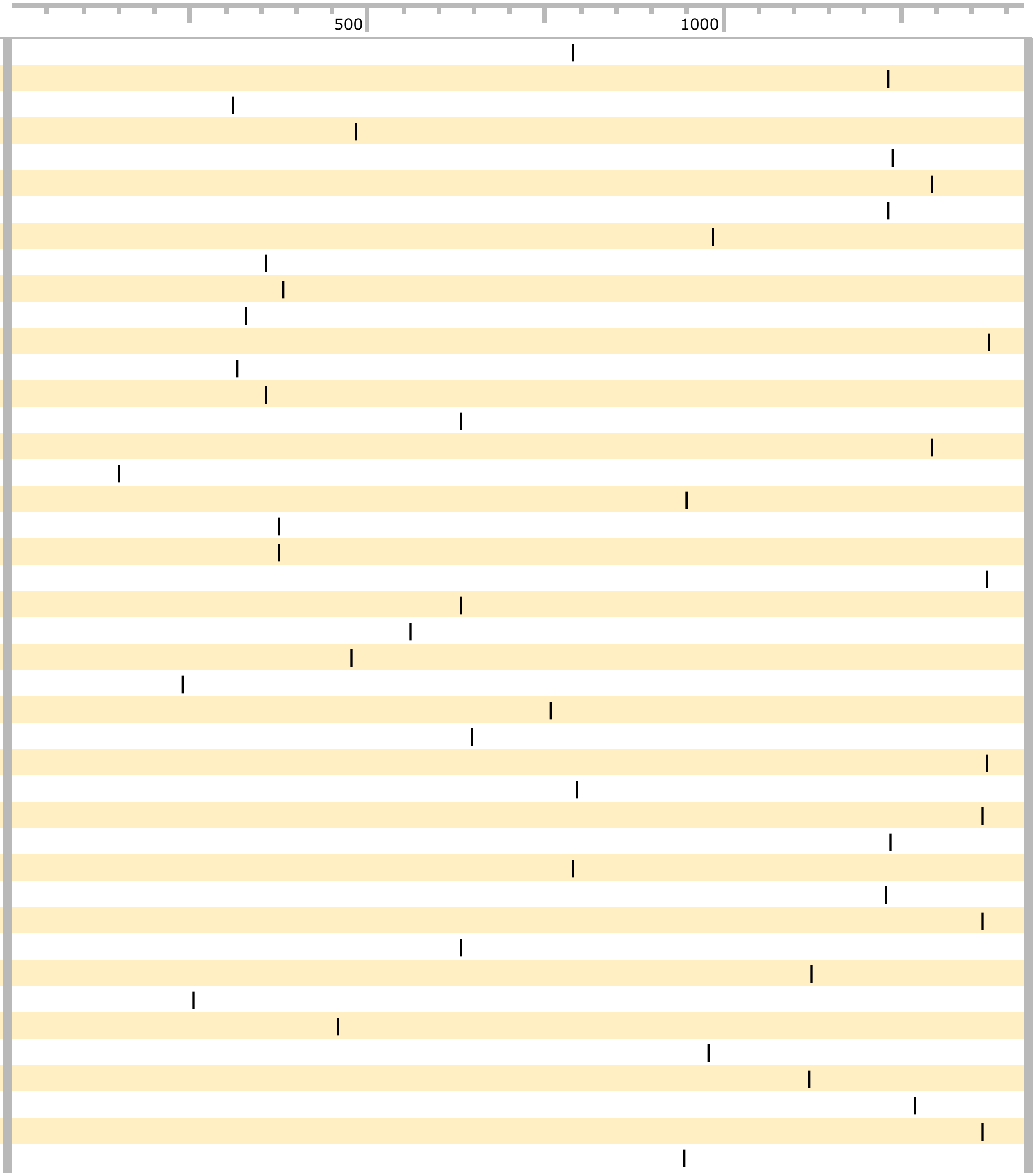
```
        XbaI                                BanI  BsrDI
CACCTTATCTAGACAACGAGAAAAGCAATGGCACCATTATCCATGTTAAGGGTAA          1320
GTGGAATAGATCTGTTGCTCTTTTCGTTACCGTGGTAATAGGTACAATTCCCATT
         10             15             20             25
P  P  Y  L  D  N  E  K  S  N  G  T  I  I  H  V  K  G  K
CD28 CAR hinge (I114 – P152)

                                               XhoI
                                               PspXI      NotI
                                               PaeR7I     EagI
GCACCTGTGCCCGTCCCCGTTGTTCCCGGGTCCAAGCAAACCGTAACTCGAGCGG          1375
CGTGGACACGGGCAGGGGCAACAAGGGCCCAGGTTCGTTTGGCATTGAGCTCGCC
             30             35
  H  L  C  P  S  P  L  F  P  G  P  S  K  P   *
CD28 CAR hinge (I114 – P152)

 BsiEI                                         End  (1422)
CCGCATCGTGACTGACTGACGATCTGCCTCGCGCGTNNNNNNNNNNN   3′
                                                     1422
GGCGTAGCACTGACTGACTGCTAGACGGAGCGCGCANNNNNNNNNNN   5′
```

Enzymes	Sites	
AflIII	1	792
ApaI	1	1236
ApaLI	1	314
AseI	1	486
BamHI	1	1241
BanI	1	1295
BanII	1	1236
BclI	1*	988*
BfuAI	1	358
BpuEI	1	384
BsaHI	1	331
BsiEI	1	1376
BsiHKAI	1	318
BspMI	1	358
BspQI	1	633
BsrDI	1	1297
BsrFI	1	152
BstBI	1	951
BtsI	1	379
BtsαI	1	379
EagI	1	1373
EarI	1	633
EcoNI	1	564
HincII	1	480
MmeI	1	241
MscI	1	761
MslI	1	648
NotI	1	1373
NspI	1	796
PaeR7I	1	1367
PasI	1	1237
PciI	1	792
PspOMI	1	1232
PspXI	1	1367
SapI	1	633
ScaI	1	1127
SfcI	1	256
SspI	1	462
SwaI	1	981
TatI	1	1125
XbaI	1	1273
XhoI	1	1367
XmnI	1	947

Enzymes	Sites
AflIII	1
ApaI	1
ApaLI	1
AseI	1
BamHI	1
BanI	1
BanII	1
BclI	1*
BfuAI	1
BpuEI	1
BsaHI	1
BsiEI	1
BsiHKAI	1
BspMI	1
BspQI	1
BsrDI	1
BsrFI	1
BstBI	1
BtsI	1
BtsαI	1
EagI	1
EarI	1
EcoNI	1
HincII	1
MmeI	1
MscI	1
MslI	1
NotI	1
NspI	1
PaeR7I	1
PasI	1
PciI	1
PspOMI	1
PspXI	1
SapI	1
ScaI	1
SfcI	1
SspI	1
SwaI	1
TatI	1
XbaI	1
XhoI	1
XmnI	1



	Feature	Location	Size		⇄	Type
✓	**tac promoter**	479 .. 507	29 bp	☐	→	promoter

▶ 3 segments

/note = strong *E. coli* promoter; hybrid between the *trp* and *lac* UV5 promoters

✓	**lac operator**	515 .. 531	17 bp	🟦	⊢⊣	protein_bind

/bound_moiety = *lac* repressor encoded by *lacI*

/note = The *lac* repressor binds to the *lac* operator to inhibit transcription in *E. coli*. This inhibition can be relieved by adding lactose or isopropyl-β-D-thiogalactopyranoside (IPTG).

✓	**GST**	554 .. 1207	654 bp	🟪	→	CDS

/product = glutathione *S*-transferase from *Schistosoma japonicum*

/translation = MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPK

218 amino acids = 25.5 kDa

✓	**HRV 3C site**	1214 .. 1237	24 bp	🟪	→	CDS

/product = recognition and cleavage site for human rhinovirus 3C and PreScission proteases

/translation = LEVLFQGP

8 amino acids = 902.1 Da

✓	**CD28 CAR hinge (I114 – P152)**	1247 .. 1363	117 bp	🟧	→	CDS

/translation = IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP

39 amino acids = 4.3 kDa

Primer	Length	●	Binding Sites	⇌	Tm	Date Added