

BGH13\_B05a3-pET32-DEST

```

5'  tggcgaatgggacgcgccctgtagcggcgcattaagcgcggcggtgtggtggttacgcgcagcgtgacc
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  accgcttaccctgcgcgggacatcgccgcgtaattcgcgcgccccacaccaccaatgcgcgtcgcactgg
o
5'  gctacacttgccagcgccttagcgcgccgctcctttcgctttctcccttctccttctcgccacgttcgccc
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  cgatgtgaacggtcgcgggatcgccggcgaggaaagcgaaagaaggaaggaaagagcggtgcaagcggc
o
5'  gctttccccgtcaagctctaaatcgggggctcccttagggttccgatttagtgctttacggcacctcga
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  cgaaaggggcagttcgagattagcccccgagggaatcccaaggctaaatcacgaaatgccgtggagct

```

**Dralll**

```

5'  ccccaaaaaacttgattagggtgatgggtcacgtagtgggcatcgccctgatagacgggttttcgccct
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  ggggttttttgaactaatccactaccaagtgcacacccggtagcgggactatctgccaaaagcggga

```

**AloI'**

**AloI**

```

5'  ttgacgttggagtccacgttctttaatagtggactcttgttcctcactggaacaacactcaaccctatct
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  aactgcaacctcaggtgcaagaaattatcacctgagaacaagggttgaccttgttgtaggtgggataga

```

**Psil**

```

5'  cggctctattcttttgatattataagggatTTTgcccgatttcggcctattggttaaaaaatgagctgattta
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gccagataagaaaactaaatatccctaaacggcctaaagccggataaccaatTTTTactcgactaaat

```

```

5'  acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  tgTTTTaaattgcgcttaaaattgTTTTataattgCGaagtTaaatccaccgtgaaaagccctttac

```

```

5'  tgcgcggaaccctatttgTTTTatttttctaatacattcaaatatgtatccgctcatgagacaataacc
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  acgcgcttggggataaacaataaaaagatttatgtaagTTTTatacatagggcgagtagtctgttattgg

```

```

5'  ctgataaatgcttcaataatattgaaaaaggaagagtatgagtagtcaacatttcggtgtcgccttatt
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gactatttacgaagttattataactTTTTccttctcatactcataagttgtaaggcacagcgggaataa

```

\_\_\_\_\_ Ap

```

5'  ccctTTTTgcccattttgccttctgtTTTTgctcaccagaaacgctggtgaaagtaaagatgctg
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gggaaaaaacgccgtaaacggaaggacaaaaacgagtgggtcttTGcaccactttcattttctacgac

```

\_\_\_\_\_ Ap

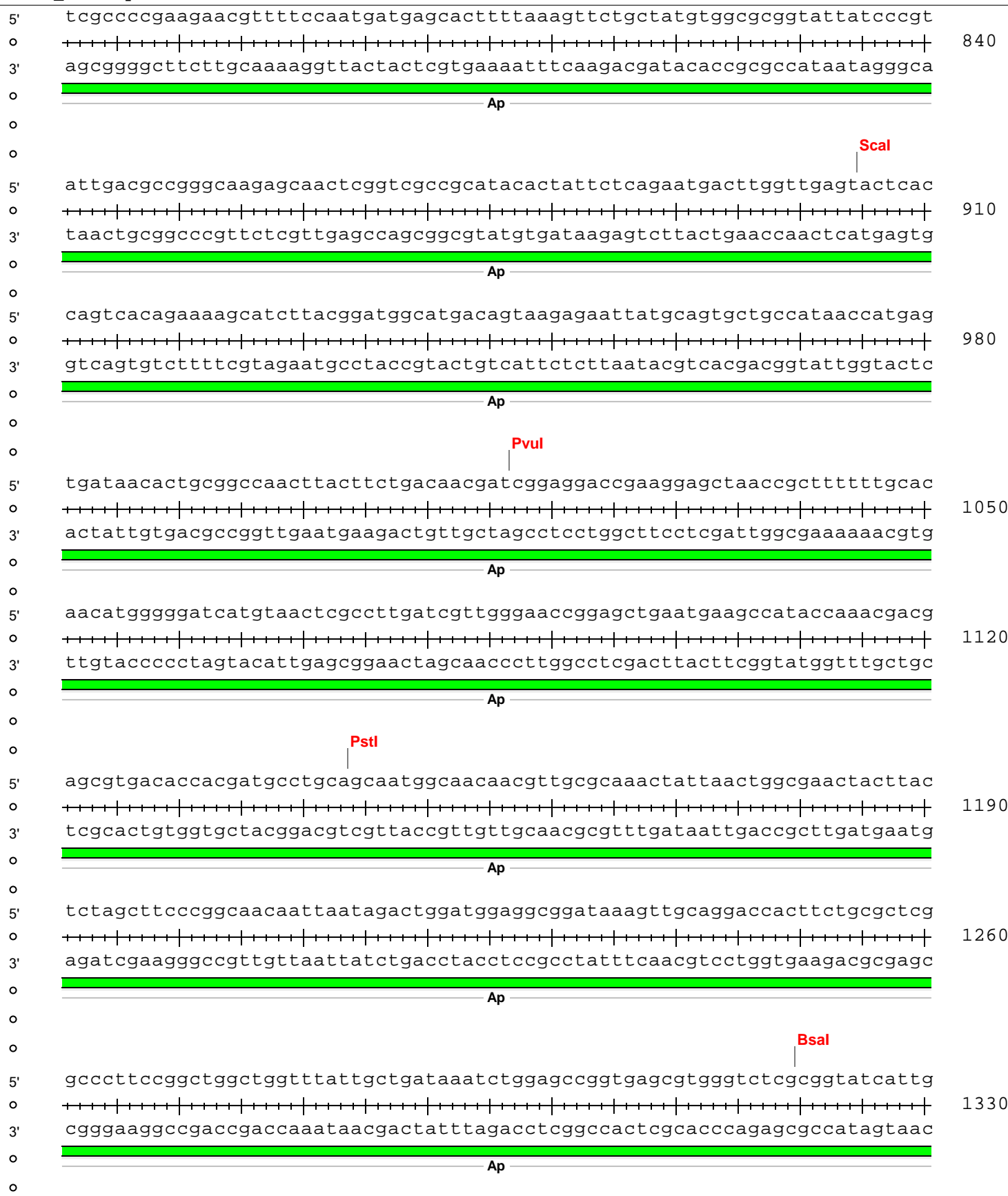
```

5'  aagatcagttgggtgcacgagtgggttacatcgaaactggatctcaacagcggtaagatccttgagagttt
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  ttctagtcaaccacgtgctcacccaatgtagcttgacctagagttgctcgccattctaggaactctcaaa

```

\_\_\_\_\_ Ap

BGH13\_B05a3-pET32-DEST



AhdI

o  
 5' cagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactat  
 o  
 3' gtcgtgacccccggtctaccattcgggagggcatagcatcaatagatgtgctgcccctcagtcctgttgata  
 o  
 Ap  
 1400

o  
 5' ggatgaacgaaatagacagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaa  
 o  
 3' cctacttgctttatctgtctagcactctatccacggagtgactaattcgtaaccttgacagtcctgggt  
 o  
 Ap  
 1470

o  
 5' gtttactcatataacttttagattgatttaaacttcatttttaatttaaaggatctaggtgaagatcc  
 o  
 3' caaatgagtatatatgaaatctaactaaattttgaaagtaaaaattaaatcttctagatccacttctagg  
 o  
 1540

o  
 5' ttttgataatctcatgaccaaatacccttaacgtgagttttcgttccactgagcgctcagaccccgtaga  
 o  
 3' aaaaactattagagtactggttttagggaattgcactcaaagcaagggtgactcgcagtcctggggcatct  
 o  
 1610

o  
 5' aaagatcaaaggatcttcttgagatcctttttctgcgcgtaatctgctgcttgcaaacaaaaaacca  
 o  
 3' tttctagtttcttagaagaactctaggaaaaaagacgcgcattagacgacgaacgtttggttttttggt  
 o  
 Ori  
 1680

o  
 5' ccgctaccagcggtggtttggttgccggatcaagagctaccaactctttttccgaaggtaactggcttca  
 o  
 3' ggcgatggctcgccaccaaacaaacggcctagtctcgcgatgggttgagaaaaggcttccattgaccgaagt  
 o  
 Ori  
 1750

o  
 5' gcagagcgcagataccaaataactgtccttctagtgtagccgtagttaggccaccacttcaagaactctgt  
 o  
 3' cgtctcgcgtctatggtttatgacaggaagatcacatcgcatcaatccgggtggtgaagttcttgagaca  
 o  
 Ori  
 1820

o  
 5' agcaccgcctacatacctcgctctgctaatacctggtaccagtggtgctgcccagtgggcgataagtcggtg  
 o  
 3' tcgtggcggatgtatggagcgagacgattaggacaatggtcaccgacgacggtcaccgctattcagcaca  
 o  
 Ori  
 1890

o  
 5' cttaccgggttgactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggggggttcgt  
 o  
 3' gaatggcccaacctgagttctgctatcaatggcctattccgcgtcgccagcccagcttgcccccgaagca  
 o  
 Ori  
 1960

o  
 5' gcacacagcccagcttgagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaag  
 o  
 3' cgtgtgtcgggtcgaacctcgcttgctggatgtggcttgactctatggatgtcgcactcgataactctttc  
 o  
 Ori  
 2030

BGH13\_B05a3-pET32-DEST

5' cgccacgcttcccgaaggggaaagggcggacaggtatccggtaagcggcagggctcggaaacaggagagcgc  
 o ++++++  
 3' gcggtgcgaagggcttccctctttccgcctgtccataggccattcgcgctcccagccttgtcctctcgcg  
 o  
 Ori  
 o

2100

5' acgagggagcttccagggggaaacgcctggtatctttatagtcctgtcggggttcgccacctctgacttg  
 o ++++++  
 3' tgctccctcgaaggtcccccttgcggaccatagaaatatcaggacagcccaaagcgggtggagactgaac  
 o  
 Ori  
 o

2170

5' agcgtcgatttttgtgatgctcgtcagggggcggagcctatggaaaaacgccagcaacgcggccttttt  
 o ++++++  
 3' tcgcagctaaaaaacactacgagcagtcccccgcctcgatacctttttgcggctcgttgcgccggaaaaa  
 o  
 Ori  
 o

2240

o  
 o  
 5' acggttctctggccttttctgctggccttttctcacatgttcttctcctgctgttatcccttgattctgtggat  
 o ++++++  
 3' tgccaaggaccggaaaaacgaccgaaaaacgagtgtacaagaaaggacgcaataggggactaagacaccta  
 o  
 PciI  
 o

2310

5' aaccgtattaccgcttttgagtgagctgataccgctcgcgcgagccgaacgaccgagcgcagcagtcag  
 o ++++++  
 3' ttggcataatggcgaaactcactcgaactatggcgagcggcgctcggcttgctggctcgcgctcgtcagtc  
 o  
 SapI  
 o

2380

5' tgagcgaggaagcggaaagagcgctgatgcggtatthctccttacgcatctgtgcggtatthcacaccg  
 o ++++++  
 3' actcgtccttctgccttctcgcggactacgccataaaagaggaatgcgtagacacgccataaagtgtggc  
 o  
 BstZ171  
 o

2450

5' catatatgggtgcaactctcagtaaatctgctctgatgcccgatagttaagccagtatacaactccgctatc  
 o ++++++  
 3' gtataataccacgtgagagtcagtttagacgagactacggcgatcaattcggtcataatgtagggcgatag  
 o  
 Tth111  
 o

2520

5' gctacgtgactgggtcatggctgcgccccgacaccgcgaacaccgctgacgcgccctgacgggcttgt  
 o ++++++  
 3' cgatgcactgaccagtagcaccgagcggggctgtgggcggttgtagggcactgagcgggactgcccgaaca  
 o

2590

5' ctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggtttcac  
 o ++++++  
 3' gacgagggccgtaggcgaatgtctgttcgacactggcagaggccctcgacgtacacagtcctccaaaagtg  
 o

2660

5' cgtcatcaccgaaacgcgcgaggcagctgcggtaaagctcatcagcgtggctcgtgaagcgattcacagat  
 o ++++++  
 3' gcagtagtggcttgcgagctccgctcgacgcccatttcgagtagtcgaccagcacttcgctaagtgtcta  
 o  
 5' gtctgctgttcatccgctccagctcgttgagtttctccagaagcgtaaatgtctggcttctgataaaag  
 o ++++++  
 3' cagacggacaagtaggcgcaggctcgagcaactcaaagaggctcttcgcaattacagaccgaagactatthc  
 o

2730

2800





BGH13\_B05a3-pET32-DEST

5' ctggtcagagacatcaagaaataacgccggaacattagtgccagcagcttccacagcaatggcatcctgg  
 0 ++++++  
 3' gaccagtctctgtagttctttattgccccttgaatcacgtccgtcgaagggtgcgttacccgtaggacc  
 0  
 0  
 0

4340

Lac I



MluI

5' tcatccagcggatagttaatgatcagcccactgacgcggttgcgcgagaagattgtgcaccgccgctttac  
 0 ++++++  
 3' agtaggtcgcctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcggcgaatg  
 0  
 0  
 0

4410

Lac I



5' aggcttcgacgcgcttcggttctaccatcgacaccaccacgctggcaccagttgatcggcgcgagattt  
 0 ++++++  
 3' tccgaagctgcccgaagcaagatggtagctgtgggtggcgcaccgtgggtcaactagccgcgctctaaa  
 0  
 0  
 0

4480

Lac I



5' aatcgccgcgacaatttgcgacggcgcggtgcagggccagactggaggtggcaacgccaatcagcaacgac  
 0 ++++++  
 3' ttagcggcgtgttaaagcgtgccgcgcacgtcccgggtctgacctccaccggttgcggtagtcggtgctg  
 0  
 0  
 0

4550

Lac I



5' tgtttgccgcagttggttgccacgcggttgggaatgtaattcagctccgccatcgccgcttccactt  
 0 ++++++  
 3' acaaacgggcggtcaacaacacggtgcgccaacccttacattaagtcgaggcggtagcggcgaagggtgaa  
 0  
 0  
 0

4620

Lac I



5' tttcccgcgttttcgcagaaacgtggctggcctgggtcaccacgcgggaaacggtctgataagagacacc  
 0 ++++++  
 3' aaagggcgcaaaagcgtctttgcaccgaccggaccaagtggtgcgccctttgccagactattctctgtgg  
 0  
 0  
 0

4690

BstAPI

|

5' ggcatactctgcgacatcgtataacggttactggtttcacattcaccaccctgaattgactctcttcggg  
 0 ++++++  
 3' ccgatatgagacgctgtagcatattgcaatgaccaaagtgtaagtgggacttaactgagagaaggccc  
 0  
 0  
 0

4760

5' cgctatcatgccataaccgcgaaaggtttgcgccattcgatgggtgtccgggatctcgacgctctccctta  
 0 ++++++  
 3' gcgatagtagcggatggcgctttccaaaacgcggtaagctaccacaggccctagagctgcgagagggaat  
 0  
 0  
 0

4830

EcoNI

|

5' tgcgactcctgcattaggaagcagcccagtagtaggtgaggccggtgagcaccgccgccaagggaatg  
 0 ++++++  
 3' acgctgaggacgtaatccttcgctcgggtcatcatccaactccggcaactcgtggcggcggcgcttccttac  
 0  
 0  
 0

4900

SphI

|

5' gtgcatgcaaggagatggcgcaccaacagtccccggccacggggcctgccaccatacccacgccgaaaca  
 0 ++++++  
 3' cacgtacgttcctctaccgcgggttgtcagggggccggtgccccggacggtggatgggtgaggcttctgt  
 0  
 0  
 0

4970

BGH13\_B05a3-pET32-DEST

5' agcgctcatgagcccgaagtggcgagcccgatcttccccatcggtgatgtcggcgatataggcgccagca 5040  
 0 ++++++  
 3' tcgcgagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt

SgrAI

5' accgcacctgtggcgccggtgatgccggccacgatgcgctccggcgtagaggatcgagatcgatctcgatc 5110  
 0 ++++++  
 3' tggcgtggacaccgcgccactacggccggtgctacgcaggccgcatctcctagctctagctagagctag

Xbal

5' ccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaataatttt 5180  
 0 ++++++  
 3' ggcgctttaattatgctgagtgatatccccttaacactcgcttattgttaaggggagatctttattaaa

T7 Promoter      Lac Operator

5' gtttaactttaagaaggagatatacatatgagcgataaaattattcacctgactgacgacagttttgaca 5250  
 0 ++++++  
 3' caaattgaaattcttcctctatatgtatactcgctattttaataagtgactgactgctgtcaaaactgt

Trx Tag

M S D K I I H L T D D S F D  
 1 2 3 4 5 6 7 8 9 10 11 12 13 14

5' cggatgtactcaaagcggacggggcgatcctcgtcgatttctgggcagagtgggtgcgggtccgtgcaaaat 5320  
 0 ++++++  
 3' gcctacatgagtttcgcctgccccgctaggagcagctaagaccgctctcaccacgccaggcacgtttta

Trx Tag

T D V L K A D G A I L V D F W A E W C G P C K M  
 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38

5' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaaactgaccgttgcaaaactgaacatc 5390  
 0 ++++++  
 3' cttagcggggctaagacctactttagcgcactgcttataagctcccgtttgactggcaacgttttgactttag

Trx Tag

I A P I L D E I A D E Y Q G K L T V A K L N I  
 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61

5' gatcaaaaccctggcactgcgcccgaatatggcatccggttatcccgactctgctgctgttcaaaaacg 5460  
 0 ++++++  
 3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

Trx Tag

D Q N P G T A P K Y G I R G I P T L L L F K N  
 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84



BGH13\_B05a3-pET32-DEST

5' gtgaagtggcggcaaccaaagtgggtgcaactgtctaaaggtcagttgaaagagttcctcgcagcctaact  
 3' cacttcaccgcccgttggtttcaccacgtagacagatttccagtcaactttctcaaggagctgcgattgga  
 Trx Tag

5530

G E V A A T K V G A L S K G Q L K E F L D A N L  
 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108

MscI

5' ggccggttctggttctggccatatgcaccatcatcatcattcttctggtctggtgccacgcggttct  
 3' ccggccaagaccaagaccggtatagctgtagtagtagtaagaagaccagaccacggtgcgccaaga  
 T...g His6-tag Thrombin

5600

A G S G S G H M H H H H H S S G L V P R G S  
 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131

BstBI

Acc65I

KpnI

5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg  
 3' ccatactttcttggcgacgacgatttaagcttgcggctcgtgtacctgtcgggtctagacctatggctgc  
 En...e

5670

G M K E T A A A K F E R Q H M D S P D L G T D  
 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154

NcoI

5' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca  
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt  
 Enterokinase attB1 TEV site

5740

BGH13\_B05a3-pDONR221

D D D K A M A I S T S L Y K K A G S E N L Y F Q  
 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178

StuI

5' aggcCTGATTGATGCCGATATGTATTGGCATATGCAGAAAGGCGATGAGAATTATGTAGTCAATCGCGGA  
 3' tccgGACTAACTACGGCTATACATAACCGTATACGTCTTTCGCTACTCTTAATACATCAGTTAGCGCCT

5810

Uknown

BGH13\_B05a3

BGH13\_B05a3-pDONR221

G L I D A D M Y W H M Q K G D E N Y V V N R G  
 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

BGH13\_B05a3-pET32-DEST

5' ATCGCTTTACATAAGATGATCCGCTTGCTGACATCTTCGACGATCAATGGCGGTTATCTGAACTTTATGG  
 3' TAGCGAAATGTATTCTACTAGGCGAACGACTGTAGAAGCTGCTAGTTACCGCCAATAGACTTGAAATACC

5880

Unknown

BGH13\_B05a3

BGH13\_B05a3-pDONR221

I A L H K M I R L L T S S T I N G G Y L N F M  
 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224

5' GTAATGAGTTTCGGGCATCCCGAATGGATCGATTTTCCGCGTGAAGGTAATGGATGGTCTTGTAAGTATGC  
 3' CATTACTCAAGCCCGTAGGGCTTACCTAGCTAAAAGGCGCACTTCCATTACCTACCAGAACATTCATACG

5950

Unknown

BGH13\_B05a3

BGH13\_B05a3-pDONR221

G N E F G H P E W I D F P R E G N G W S C K Y A  
 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248

5' CCGTCGTCAGTGGGATCTGGTAGATAACAAGAATCTGGCTTATCACTACATGGGGGACTTTGATGCTGAG  
 3' GGCAGCAGTCACCCTAGACCATCTATTGTTCTTAGACCGAATAGTGATGTACCCCTGAAACTACGACTC

6020

Unknown

BGH13\_B05a3

BGH13\_B05a3-pDONR221

R R Q W D L V D N K N L A Y H Y M G D F D A E  
 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271

5' ATGCTGAAAGTGATCAAGAGTGTGAAAGATTTCCAGGCGACTCCGGTCCAGGAGATCTGGCATAATGATG  
 3' TACGACTTTCACTAGTTCTCACACTTTCTAAAGGTCCGCTGAGGCCAGGTCTCTAGACCGTATTACTAC

6090

Unknown

BGH13\_B05a3

AmyC beta sheets

BGH13\_B05a3-pDONR221

M L K V I K S V K D F Q A T P V Q E I W H N D  
 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294

5' GTGATCAGGTATTGGCTTATGGACGTAAGGATCTGATATTTGTATTTAACTTCAATCCGAAACAGTCTTT  
 3' CACTAGTCCATAACCGAATACCTGCATTCTAGACTATAAACATAAATTGAAGTTAGGCTTTGTCAGAAA

6160

BGH13\_B05a3

AmyC beta sheets

BGH13\_B05a3-pDONR221

G D Q V L A Y G R K D L I F V F N F N P K Q S F  
 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318

BGH13\_B05a3-pET32-DEST

5' TGTAGATTATGGTTTCCTCGTTAGTCCGGGAGCTTATGAAGTAATCTTGAATACGGATAATGTTGCATTT  
 3' ACATCTAATAACCAAAGGAGCAATCAGGCCCTCGAATACTTCATTAGAACTTATGCCTATTACAACGTAAA

6230

BGH13\_B05a3

AmyC beta sheets

BGH13\_B05a3-pDONR221

V D Y G F L V S P G A Y E V I L N T D N V A F  
 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

5' GGCGGAAATGGTCTTGCAGATGATTCAGTAGTTCATTTACGATTGCCGATCCGTTGTATAAGAAGGAAA  
 3' CCGCCTTTACCAGAACGTCTACTAAGTCATCAAGTAAAGTGCTAACGGCTAGGCAACATATTCTTCCTTT

6300

BGH13\_B05a3

AmyC beta sheets

BGH13\_B05a3-pDONR221

G G N G L A D D S V V H F T I A D P L Y K K E  
 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364

5' AGAAAGAGTGGCTGAAACTCTATATTCCTGCACGTACAGCTGTTGTTTTGAGAAAGAAGAAATAGGACCC  
 3' TCTTTCTCACCGACTTTGAGATATAAGGACGTGCATGTCGACAACAAAACCTCTTCTTTCTTTATCCTGGG

6370

BGH13\_B05a3

AmyC beta sheets

BGH13\_B05a3-pDONR221

K K E W L K L Y I P A R T A V V L R K K K  
 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386

5' AGCTTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgctcgacaagcttgcggccgcact  
 3' TCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacgccggcgtga

6440

BamHI

EcoRI

EcoRI  
SacI

SalI

HindIII

NotI  
EagI

PspXI  
XhoI  
AvaI

attB2

B...1

BmeT110I

5' cgagcaccaccaccaccactgagatccggctgctaacaaagcccgaaaggaagctgagttggctgct  
 3' gctcgtgggtgggtgggtgactctaggccgacgattgttccgggctttccttcgactcaaccgacga

6510

BplI

5' gccaccgctgagcaataactagcataaaccccttggggcctctaaacgggtccttgaggggttttttgcctga  
 3' cgggtggcgactcgttattgatcgtattggggaaccccgagatttgcccagaactccccaaaaaacgact

6580

T7 Terminator

5' aaggaggaactatatccggat  
o +++++|++++|++++|++++|+  
3' ttctccttgatataggccta  
o

6601