

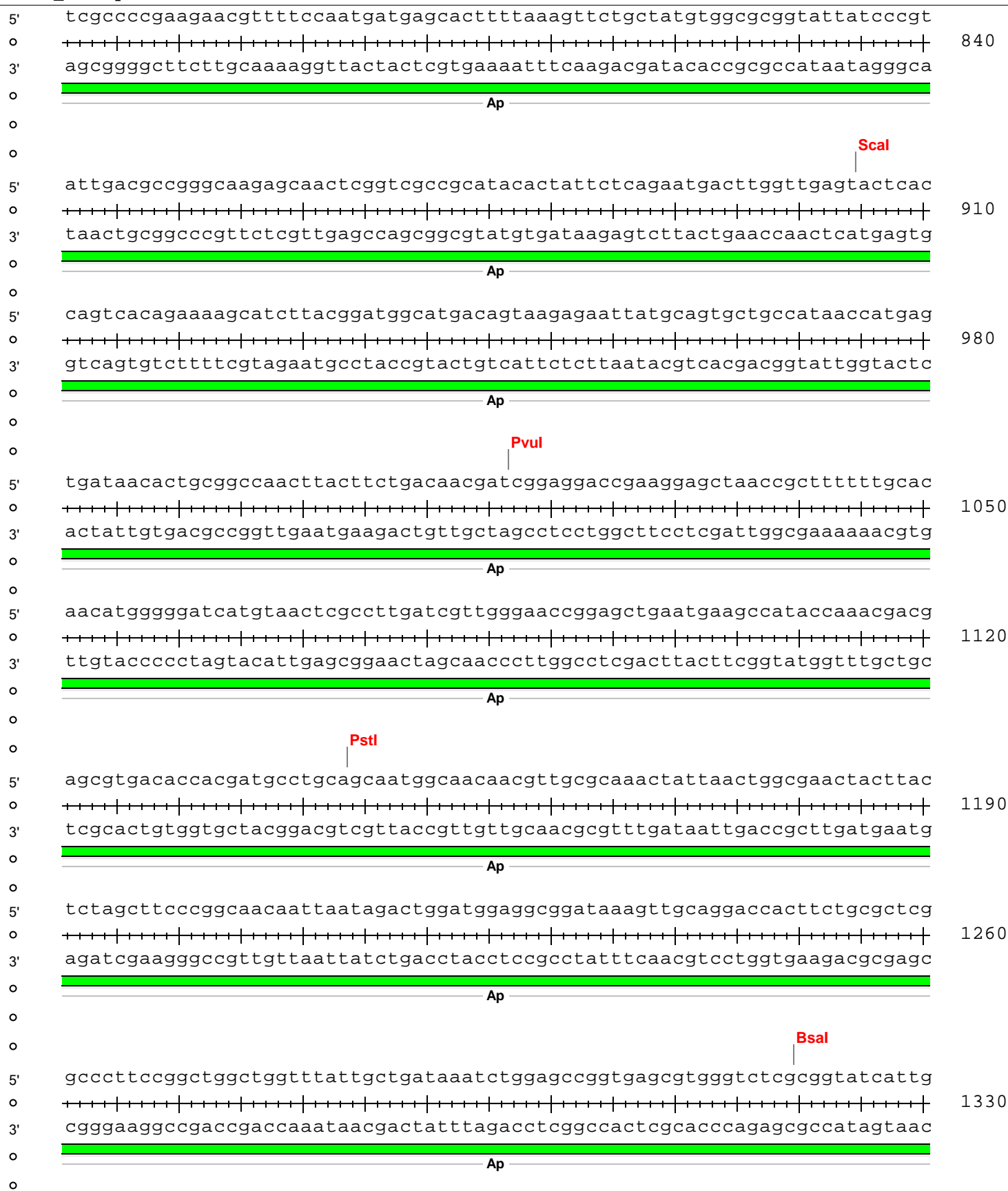
BGT25_T02c-pET32-DEST

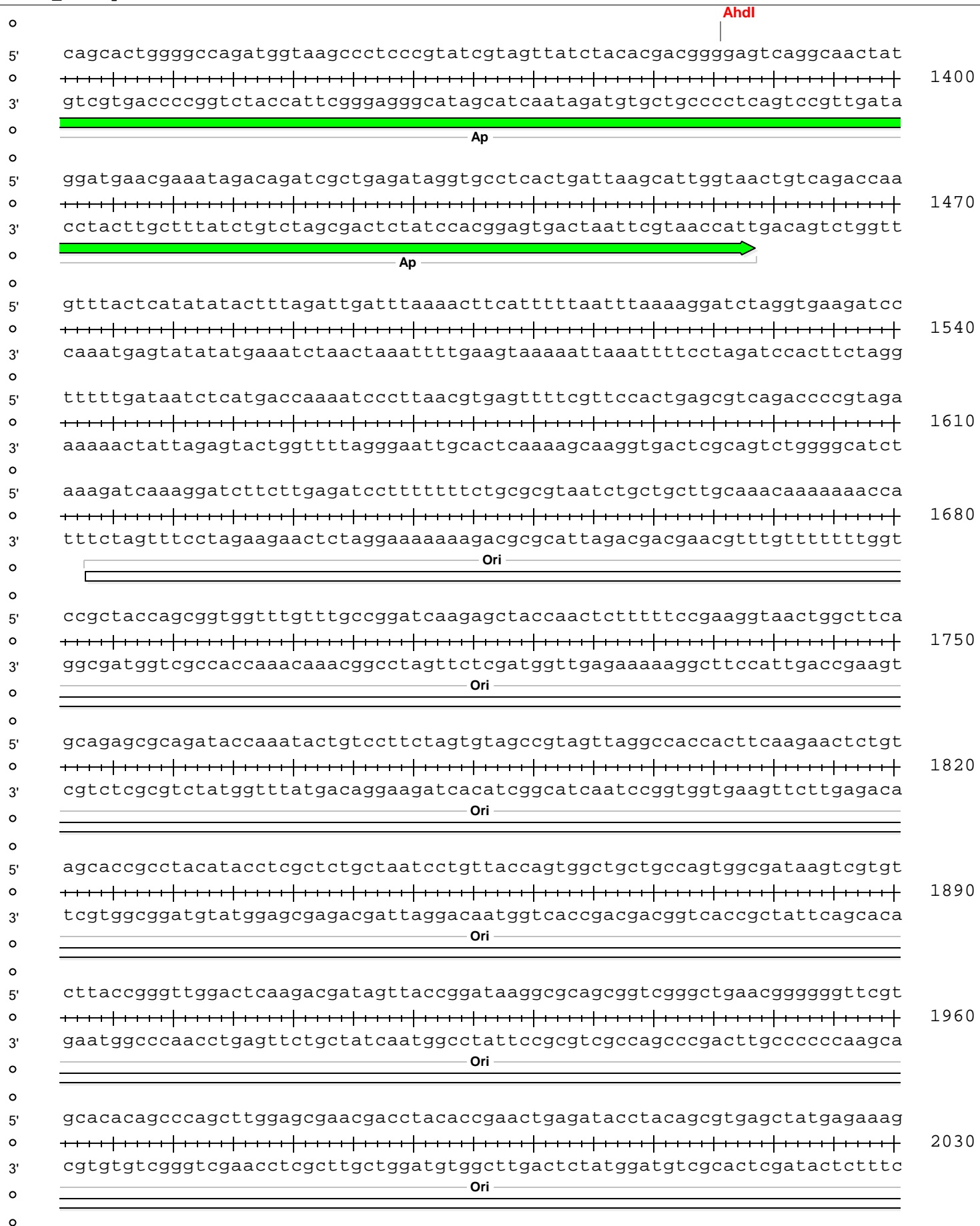
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5'  tggcgaatgggacgcgccctgtagcggcgccattaagcgcggcggggtggtggttacgcgcagcgtgacc
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  accgcttaccctgcgcggggacatcgccgcgtaattcgcgccgccacaccaccaatgcgcgtcgcaactgg
o
5'  gctacacttgccagcgccttagcgcgccgctcctttcgctttcttcccttcttctcgcaccgttcgccg
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  cgatgtgaacgggtcgcgggatcgcggggcaggaaagcgaagaaggaaggaaagagcgggtgcaagcggc
o
5'  gctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcga
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  cgaaaggggcagttcgagatttagccccgagggaatcccaaggctaaatcacgaaatgccgtggagct
o
                    Dralll
                    |
5'  ccccaaaaaacttgattaggggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccct
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  ggggttttttgaactaatccactaccaagtgcataccccggtagcgggactatctgccaaaagcggga
o
Aol'              Aol
|                 |
5'  ttgacggtggagtccacgcttcttaaatagtgactcttgttccaaactggaacaacactcaaccctatct
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  aactgcaacctcaggtgcaagaaattatcacctgagaacaaggtttgaccttgttgtgagttgggataga
o
                    Psil
                    |
5'  cggctctattcttttgatttataagggattttgccgatttcggcctattgggttaaaaaatgagctgattta
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gccagataagaaaactaaatatccctaaaacggctaaagccggataaccaattttttactcgactaaat
o
5'  acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  tgtttttaaattgcgcttaaaattgttttataattgCGAATgttaaatccaccgtgaaaagcccttttac
o
5'  tgcgcggaaccctatttgtttatttttctaatacattcaaatatgtatccgctcatgagacaataacc
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  acgcgccttggggataaacaaaataaaaagatttatgtaagtttatacatagggcagtagtactctgttattgg
o
5'  ctgataaatgcttcaataatattgaaaaaggaagagtatgagattcaacatttcggtgctgcccttatt
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gactatttacgaagtattataactttttccttctcatactcataagttgtaaaaggcacagcgggaataa
o
                    _____
                    |
                    Ap
o
5'  cccttttttgcggcattttgccttctgtttttgctcaccagaaacgctggtgaaagtaaagatgctg
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3'  gggaaaaaacgccgtaaaacggaaggacaaaaacgagtggtctttgcgaccactttcattttctacgac
o
                    _____
                    |
                    Ap
o
5'  aagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttt
o  +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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o
                    _____
                    |
                    Ap
o

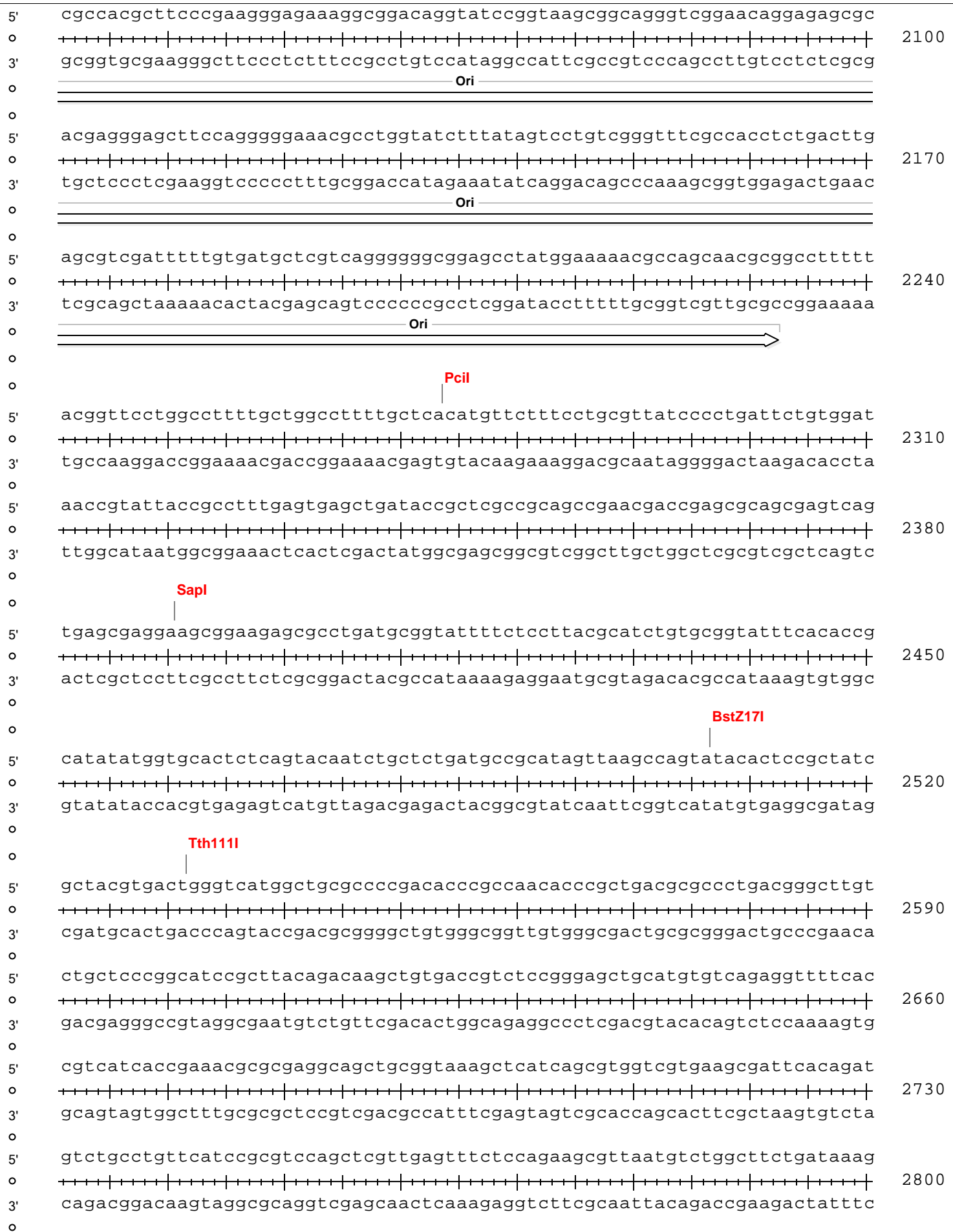
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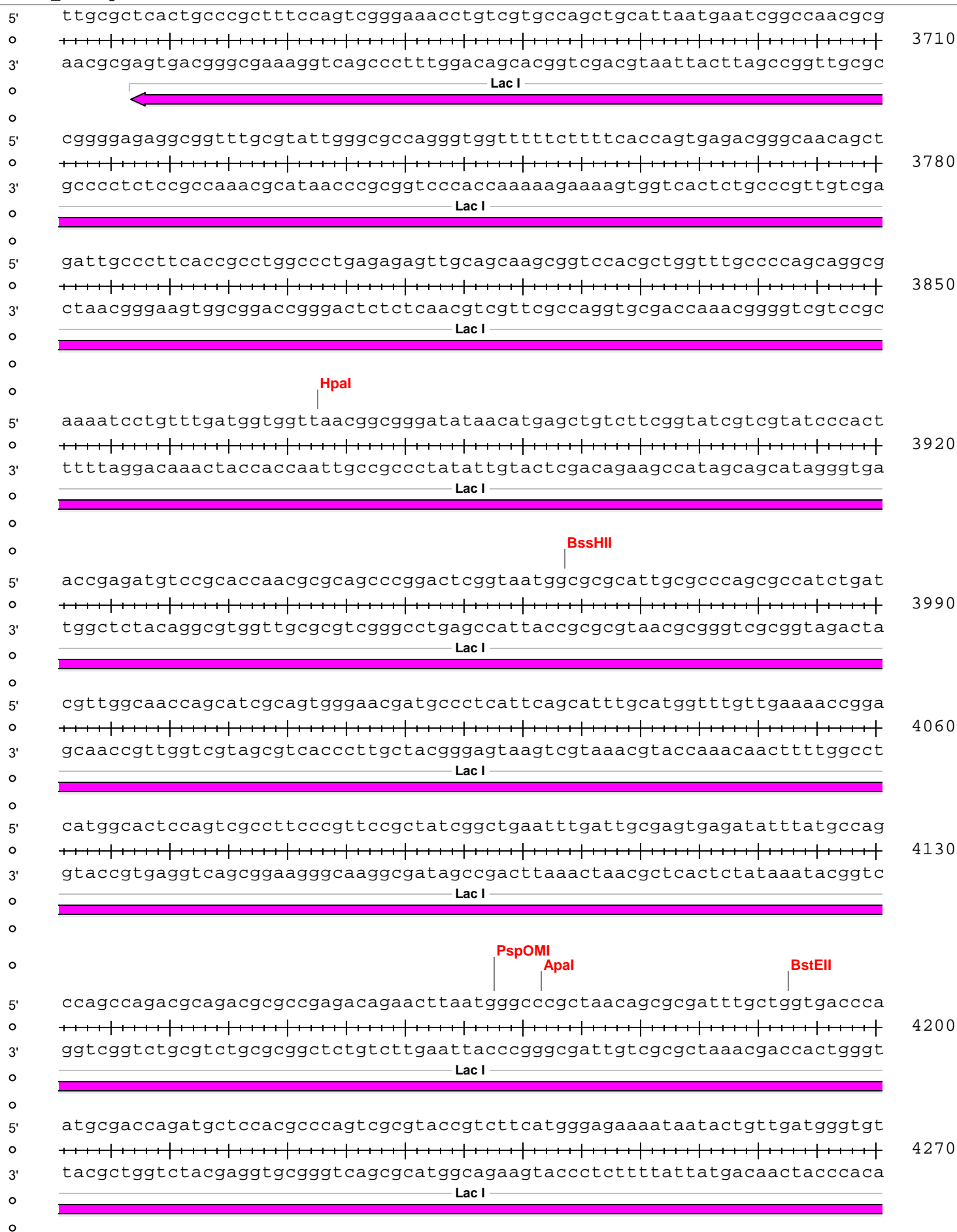




BGT25_T02c-pET32-DEST



BGT25_T02c-pET32-DEST



5' agcgcctcatgagcccgaagtggcgagcccgatcttccccatcggtgatgtcggcgatataggcgccagca
 ++++|||||
 3' tcgcgagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt

5040

SgrAI

5' accgcacctgtggcgccggtgatgccggccacgatgctcggcgtagaggatcgagatcgatctcgatc
 ++++|||||
 3' tggcgtggacaccgcggccactacggccggtgctacgcaggccgcattctcctagctctagctagagctag

5110

XbaI

5' ccgcgaaattaatacgaactcactataggggaattgtgagcggataacaattcccctctagaataatttt
 ++++|||||
 3' ggcgctttaattatgctgagtgatatccccttaacactcgctattgttaaggggagatctttattaaa

5180

T7 Promoter

Lac Operator

BamI

BamI'

5' gtttaactttaagaaggagatatcatatgagcgataaaattattcacctgactgacgacagtttggaca
 ++++|||||
 3' caaattgaaattcttccctctatatgtatactcgctattttaataagtgactgactgctgtcaaaactgt

5250

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

RsrII

5' cggatgtactcaaagcggacggggcgatcctcgctcgatttctgggcagagtgggtgcggtccgtgcaaaat
 ++++|||||
 3' gcctacatgagtttcgcctgccccgctaggagcagctaagaccgctctcaccacgccaggcagctt

5320

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' gatcgcgccgattctggatgaaatcgctgacgaatatcaggggcaaaactgaccggttgcaaaaactgaacatc
 ++++|||||
 3' cttagcggggctaagacctacttttagcgactgcttatagtcccgtttgactggcaacgttttgactttag

5390

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' gatcaaaaccctggcactgcgcgcaaatatggcatccggttatcccgactctgctgctgttcaaaaacg
 ++++|||||
 3' ctagttttgggaccgtgacgcggctttataaccgtaggcaccatagggttgagacgacgacaagtttttgc

5460

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

BGT25_T02c-pET32-DEST

5' gtgaagtggcggcaaccaaagtgggtgcaactgtctaaaggtcagttgaaagagttcctcgacgctaacct
 3' cacttcaccgcccgttggtttcaccacgtgacagatttccagtcaactttctcaaggagctgcgattgga
 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' ggccggttctgggttctggccatatgcaccatcatcatcattcttctgggtctgggtgccacgcggttct
 3' ccggccaagaccaagaccggtatacgtggtagtagtagtaagaagaccagaccacgggtgcgccaaga
 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 BglIII Acc65I KpnI
 5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg
 3' ccatacttcttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacctatggctgc
 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

5' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt
 Enterokinase attB1 TEV site
 BGT25_T02c-pDONR221

5740

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

5' aggcCGTGTTTTTTATCATTCTTTAAATCAAAAAGTGTGCGATAAATTTGGTTTGGTTTTTATAGAGACACC
 3' tccgGCACAAAAATAGTAAAGAAATTTAGTTTTTTCACACGCTATTTAAACCAAAACCAAAAATCTCTGTGG
 T... BGT25_T02c
 BGT25_T02c-pDONR221

5810

G R V F I I S L N Q K V C D K F G L V F R D T
 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

BGT25_T02c-pET32-DEST

5' ACAACTTTACTCAATAATATCAACGCCACCCACCACCAAGCGCAAATTTTTGATGCGATTTATTCCAAGA
 0 ++++++
 3' TGTTGAAATGAGTTATTATAGTTGCGGTGGGTGGTTCGCGTTTAAAACTACGCTAAATAAGGTTCT

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----- BGT25_T02c-pDONR221 -----

5880

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

5' CTTTTGAAGGCGGGTTGCACCCCTAGTTAAAAAGCATTTACACCCTTATTTTCATCACGCAAAATATCAA
 0 ++++++
 3' GAAAAC TTCGCCAACGTGGGGGATCAATTTTTCGTAAATGTGGGAATAAAGTAGTGCGT TTTATAGTT

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5950

T F E G G L H P L V K K H L H P Y F I T Q N I K
 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' AGACATGGGGATTGCAACCAGTCTCATCAGTGAGTTTCTAAGTTTATTACGCTTTAAAAATACCATGCG
 0 ++++++
 3' TCTGTACCCCTAACGTTGGTCAGAGTAGTCACTCCAAAGATTCAAATAATGCGAAATTTTATGGTACGC

----- BGT25_T02c -----

----- BGT25_T02c-pDONR221 -----

6020

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

5' AAATTTATGAGCTTGGGGGAGCTTGGGTGCTATGCGAGCCATTATTCGTTGTGGCAAAAATGCATAGAGC
 0 ++++++
 3' TTAAATACTCGAACCCCTCGAACCCACGATACGCTCGGTAATAAGCAACACCGTTTTTTACGTATCTCG

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----- BGT25_T02c-pDONR221 -----

6090

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

5' TCAATGAAGCGATCTGTATTTTAGAAGACGATATAACCTTGAAAGAGGATTTTAAAGAGGGCTTGGATTT
 0 ++++++
 3' AGTTACTTCGCTAGACATAAAATCTTCTGCTATATTGGAAC TTTCTCCTAAAATTTCTCCCGAACCTAAA

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----- BGT25_T02c-pDONR221 -----

6160

L N E A I C I L E D D I T L K E D F K E G L D 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

BGT25_T02c-pET32-DEST

5' TTTAGAAAAACACATCCAAGAGTTAGGCTATGCGCGTTTGATGCATTTATTGTATGATGCCAGCGTGAAA
 3' AAATCTTTTTGTGTAGGTTCTCAATCCGATACGCGCAAACACTACGTAAATAACATACTACGGTCGCACTTT

6230

BGT25_T02c

BGT25_T02c-pDONR221

L E K H I Q E L G Y A R L M H L L Y D A S V K
 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

5' AGTGAGCCTTTAAACCATAAAAAACCAAGAGATAACAAGAGCGTGTGGGATCATTAAGCTTATAGCCATG
 3' TCACTCGGAAATTTGGTATTTTGGTTCTCTATGTTCTCGCACACCCCTAGTAATTTCGAATATCGGTAC

6300

BGT25_T02c

BGT25_T02c-pDONR221

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

5' GGGTGGGGACGCAAGGCTATGTGATCACGCCAAGATTGCCAAAGTTTTTTTGAACACAGCCGAAAATG
 3' CCCACCCCTGCGTTCGGATACACTAGTGC GGTTCTAACGGTTTCAAAAAACTTTGTGTCGGCTTTTAC

6370

BGT25_T02c

BGT25_T02c-pDONR221

G V G T Q G Y V I T P K I A K V F L K H S R K W
 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388

5' GGTTGTTCTGTGGATACGATAATGGACGCTACTTTTATCCATGGCGTGAAAAATCTGGTGTTACAACCT
 3' CCAACAAGGACACCTATGCTATTACCTGCGATGAAAATAGGTACCGCACTTTTGTAGACCACAATGTTGGA

6440

BGT25_T02c

BGT25_T02c-pDONR221

V V P V D T I M D A T F I H G V K N L V L Q P
 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411

5' TTTGTGATCGCTGATGATGAGCAAATCTCTACGATAGCGCGAAAAGAAGAACCCCTATAGCCCTAAAATCG
 3' AAACACTAGCGACTACTACTCGTTTAGAGATGCTATCGCGCTTTTCTTCTTGGGATATCGGGATTTTAGC

6510

BGT25_T02c

BGT25_T02c-pDONR221

F V I A D D E Q I S T I A R K E E P Y S P K I
 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434

BGT25_T02c-pET32-DEST

