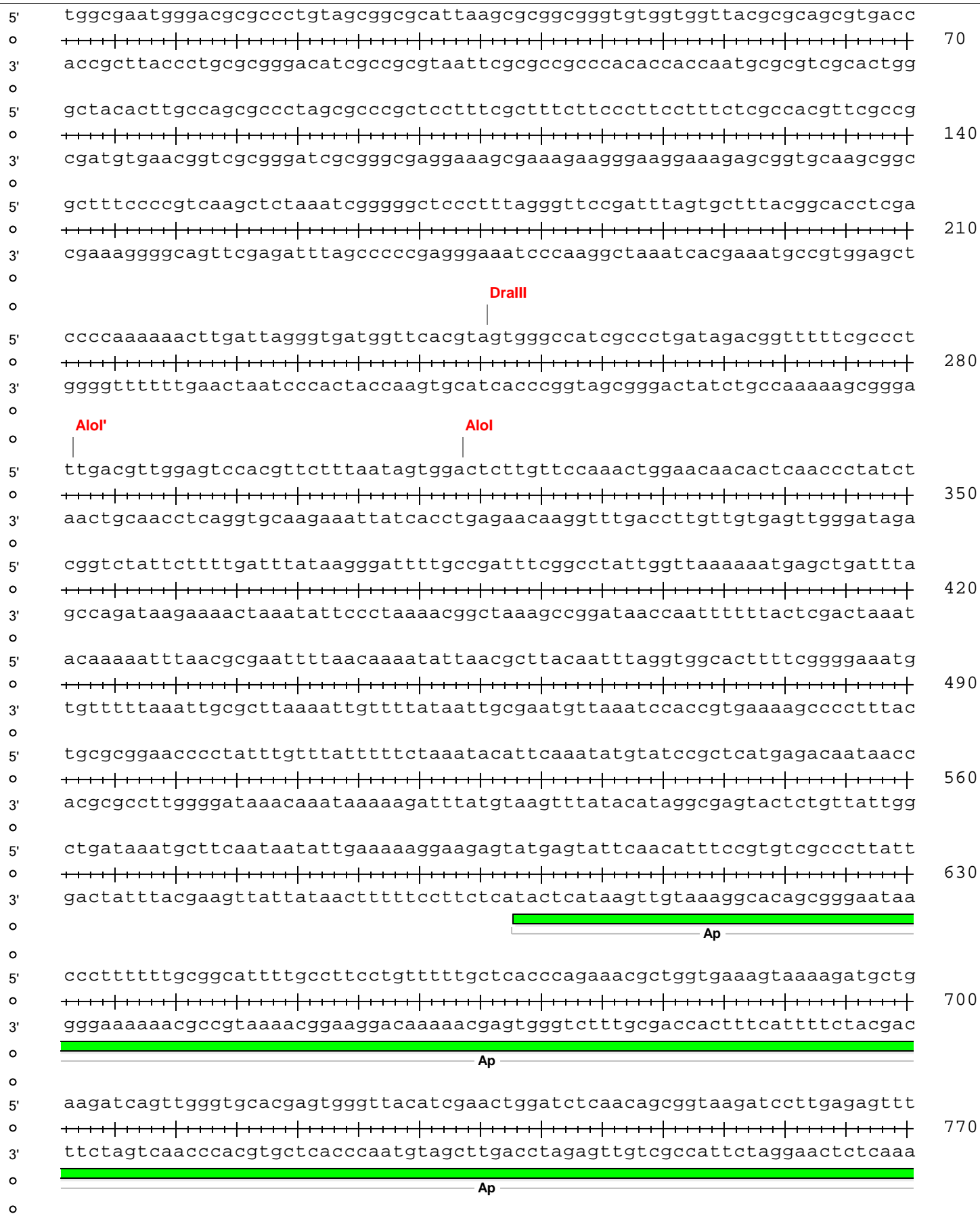
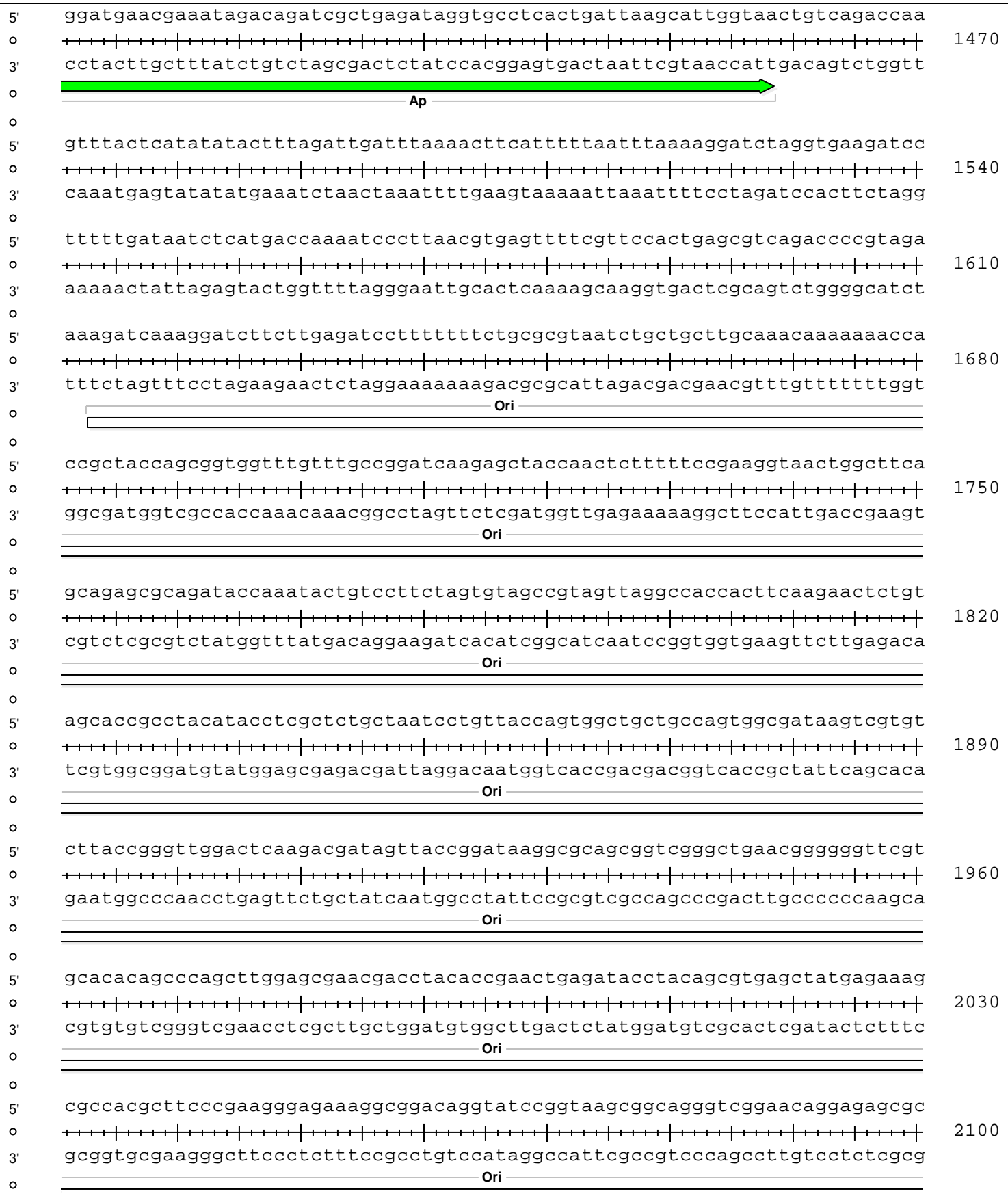


BGT27_T18a-pET32-DEST



BGT27_T18a-pET32-DEST

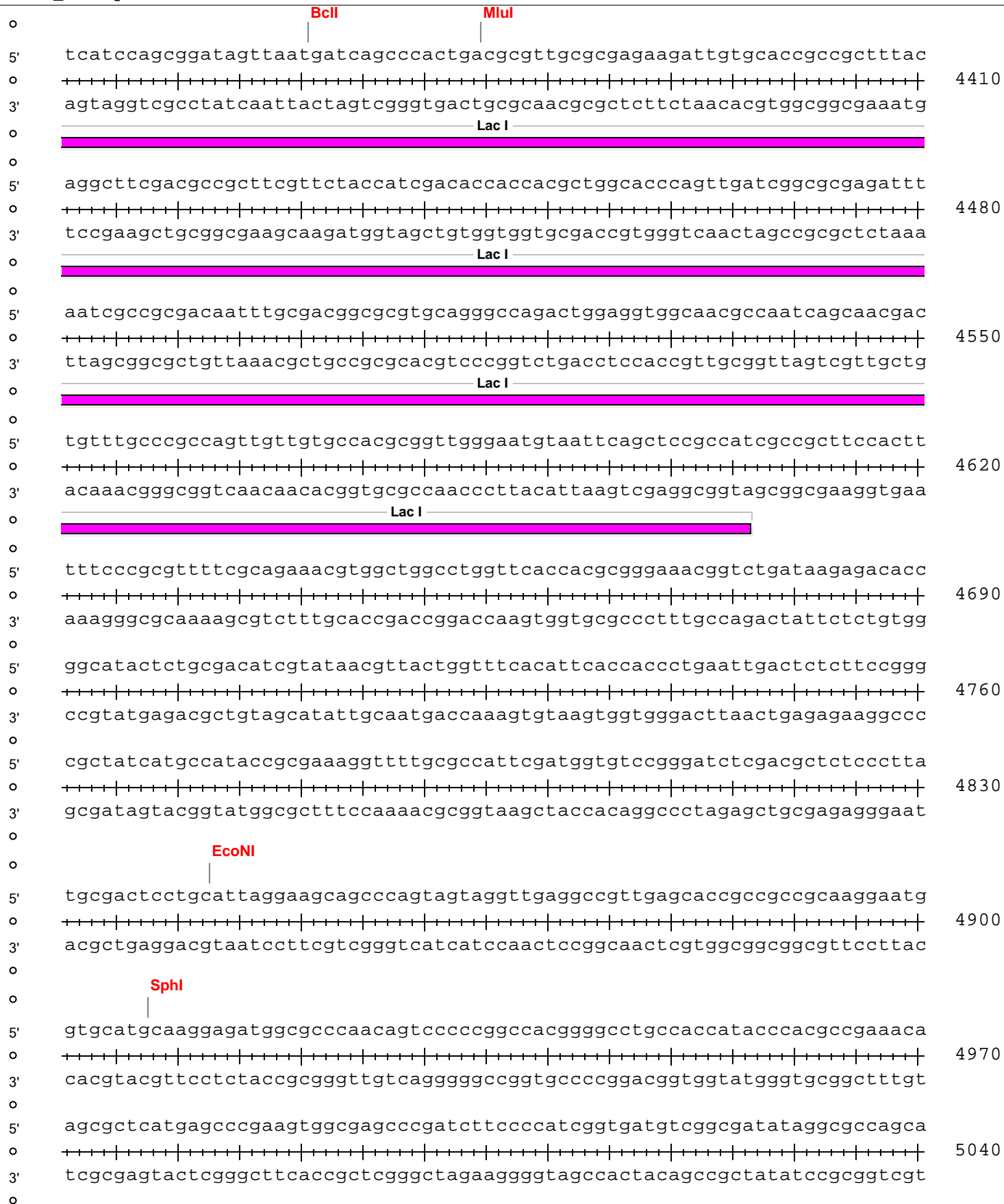


BGT27_T18a-pET32-DEST

```

5'  acgagggagcttccaggggaaacgcctggtatctttatagtcctgtcggggttcgccacctctgacttg
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  tgctccctcgaaggcccccttgcggaccatagaaatatcaggacagccaaagcgggtggagactgaac
o  ===== Ori =====
o  =====
5'  agcgtcgatTTTTgtgatgctcgtcagggggcggagcctatggaaaaacgccagcaacgcggccttttt
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  tcgcagctaaaaaactacgagcagtcccccgcctcgatacctTTTTgcggtcgttgcgccggaaaaaa
o  ===== Ori =====>
o  =====
o  PciI
5'  acggttcttggccttttgctggccttttgctcacatggttctttcctgcgttatccctgattctgtggat
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  tgccaaggaccggaaaaacgaccggaaaaacgagtgtacaagaaaggacgcaataggggactaagacaccta
o  =====
5'  aaccgtattaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgagtcag
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  ttggcataatggcggaaactcactcgactatggcgagcggcgtcggttgctggctcgcgctcgctcagtc
o  =====
o  Sapl
5'  tgagcgaggaagcggaaagagcgcctgatgcggtattttctccttacgcatctgtgcggtatttcacaccg
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  actcgctccttcgcttctcgcggactacgccataaaagaggaatgcgtagacacgccataaagtgtggc
o  =====
o  BstZ171
5'  catatatggtgcactctcagtacaatctgctctgatgccgatagttaagccagtatacactccgctatc
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  gtataataccacgtgagagtcattgtagacgagactacggcgtatcaattcggtcatatgtgaggcgatag
o  =====
o  Tth111I
5'  gctacgtgactgggtcatggctgcgcctccgacacccgccaacacccgctgacgcgccctgacgggcttgt
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  cgatgcaactgaccagtagcagcgcgggctgtgggcggttgtgggcgactgcgcgggactgcccgaaca
o  =====
5'  ctgctcccggcatccgcttacagacaagctgtgaccgtctccgggagctgcatgtgtcagaggTTTTcac
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  gacgagggccgtagggcaatgtctgttcgacactggcagaggccctcgacgtacacagttccaaaagtg
o  =====
5'  cgtcatcaccgaaacgcgcgagggcagctgcggtaaagctcatcagcgtggctcgtgaagcgattcacagat
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  gcagtagtggcttgcgcgctccgtcgacgccatttcgagtagtcgcaccagcacttcgctaagtgtcta
o  =====
5'  gtctgcctgttcacccgcgtccagctcgttgagtttctccagaagcgttaatgtctggcttctgataaaag
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  cagacggacaagtagggcgcaggtcgagcaactcaaagaggtcttcgcaattacagaccgaagactatTTC
o  =====
5'  cgggccatgttaagggcgggtTTTTcctgttggctcactgatgcctccgtgtaagggggatttctgttca
o  +++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3'  gcccgtacaattcccgccaaaaaaggacaaaccagtgactacggaggcacattccccctaagacaagt
o  =====

```

o
5' accgcacctgtggcgccgggtgatgccggccacgatgctccggcgtagaggatcgagatcgatctcgatc
o
3' tggcgtggacaccgcgccactacggccggtgctacgcaggccgcacatctcctagctctagctagagctag
o
5110

SgrAI

XbaI

o
5' ccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaataatttt
o
3' ggcgctttaattatgctgagtgatatccccttaacactcgctattgttaaggggagatctttattaaa
o
T7 Promoter Lac Operator

o
5' gtttaactttaagaaggagatatacatatgagcggataaaattattcacctgactgacgacagttttgaca
o
3' caaattgaaattcttcctctatatgtatactcgctattttaataagtgactgactgctgtcaaaactgt
o
Trx Tag

BamI

BamI'

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

o
5' cggatgtactcaaagcggacggggcgatcctcgtcgatttctgggcagagtggtgctgggtccgtgcaaaat
o
3' gcctacatgagtttcgctgccccgctaggagcagctaaagaccgctctcaccacgccaggcaggtttta
o
Trx Tag

RsrII

o
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
o
5' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaactgaccgttgcaaaactgaacatc
o
3' ctagcggggctaagacctactttagcgcactgcttatagtcccgtttgactggcaacgttttgacttgtag
o
Trx Tag

o
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
o
5' gatcaaaaccctggcactgcgccgaaatatggcatccgtggatcccgactctgctgctgttcaaaaacg
o
3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc
o
Trx Tag

o
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

BGT27_T18a-pET32-DEST

5' gtgaagtggcggcaaccaaagtgggtgcaactgtctaaagggtcagttgaaagagttcctcgacgctaact
 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' ggccggttctggttctggccatatgcaccatcatcatcattcttctggtctggtgccacgcggttct
 3' ccggccaagaccaagaccggtatacgtggtagtagtagtaagaagaccagaccacggtgcgccaaga
 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

BgIII

Acc65I

KpnI

5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg
 3' ccatacttcttggcgacgacgatttaagcttgcggtcgtgtacctgctcggtctagacctatggctgc
 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' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtaactttca
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt
 Enterokinase attB1 TEV site

5740

BGT27_T18a-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

5' aggcAATAATTCAAATTGACTAAAACATCGATTATTATCCCCTGTAAAAATGAAGGAATTTATGTTAAA
 3' tccgTTATTAAGTTTTAACTGATTTTGTAGCTAATAATAGGGGACATTTTACTTCCTTAAATACAATTT
 BGT27_T18a

5810

BGT27_T18a-pDONR221

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

BGT27_T18a-pET32-DEST

5' CAGACTATAGAGTTTTTTATATAGAAGCTGAAGCTAAGTATATATCAACTATAATCGTCATTAATGATAACT
 0 ++++++
 3' GTCTGATATCTCAAAAATATATCTTGACTTCGATTCATATATAGTTGATATTAGCAGTAATTACTATTGA

5880

BGT27_T18a

BGT27_T18a-pDONR221

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

5' CTAACGATAATTGTTGTGAATTTTTGAAAAATCCTTTTAACAAATTTTCAAAGGTAAAATTGATAGAAAC
 0 ++++++
 3' GATTGCTATTAACAACACTTAAAACTTTTGTAGGAAAATTGTTTAAAAGTTTCCATTTTAACTATCTTTG

5950

BGT27_T18a

BGT27_T18a-pDONR221

S N D N C C E F L K N P F N K F S K V K L I E T
 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' TACTGGCATAGGAGCAGCCGCAGCAAGAAATCTTGGGGCAACCTTTGCAGAAAACCTCAGAAATATTAGTT
 0 ++++++
 3' ATGACCGTATCCTCGTCGGCGTCTTTAGAACCCCGTTGGAAACGTCTTTTGAGTCTTTATAATCAA

6020

BGT27_T18a

BGT27_T18a-pDONR221

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

5' TTCTGTGATGCTCATATTACAATGAACCAAAATTGGCTTAATATTCTTTTATCTGCCTTTAATGATAAAA
 0 ++++++
 3' AAGACACTACGAGTATAATGTTACTTGGTTTTAACCGAATTATAAGAAAATAGACGGAAATTACTATTTT

6090

BGT27_T18a

BGT27_T18a-pDONR221

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

Sbfl

5' ATGTAAGTGTAATTTGCCAGGTATCGGCCATTTTTCTCCTGCTAGTCCTGCAGGCTATGGTCAAAGCTT
 0 ++++++
 3' TACATTCACATTAACGGGTCCATAGCCGGTAAAAAGAGGACGATCAGGACGTCCGATAACCAGTTTCGAA

6160

BGT27_T18a

BGT27_T18a-pDONR221

N V S V I C P G I G H F S P A S P A G Y G 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

BGT27_T18a-pET32-DEST

SpeI

5' TAATGAGAGCTTTGAAACATATTGGCTAAAAAAGCCACTGAATATAAAAGAAGTTCCTACTAGTTCCTGGT
 3' ATTACTCTCGAAACTTTGTATAACCGATTTTTTTCGGTGACTTATATTTTCTTCAAGGTGATCAAGGACCA

6230

BGT27_T18a

BGT27_T18a-pDONR221

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

5' GGTTGCATGGCTATAAAAAAGAAACCTTTGATGCAGTAGACGGCTTTGATCGAGGTTTCCATAGTTGGG
 3' CCAACGTACCGATATTTTTTCTTTGGAACTACGTCATCTGCCGAACTAGCTCCAAAGGTATCAACCC

6300

BGT27_T18a

BGT27_T18a-pDONR221

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

5' GCTTTGAAGATGTAGAACTTTCTATAAAACTCTGGCTTTTCGGATATAAAATTTTTGTACATCCTGCTGT
 3' CGAAACTTCTACATCTTGAAAGATATTTTGAGACCGAAAAGCCTATATTTTAAAAACATGTAGGACGACA

6370

BGT27_T18a

BGT27_T18a-pDONR221

G F E D V E L S I K L W L F G Y K I F V H P A V
 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' TAAAGTTGGACACAAATTTAGAAAAATACAACCCTATGATGTTGACCTAACGGAATTCATTACAATAAA
 3' ATTTCAACCTGTGTTTAAATCTTTTTATGTTGGGATACTACAACCTGGATTGCCTTAAGGTAATGTTATTT

6440

BGT27_T18a

BGT27_T18a-pDONR221

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

SnaBI

5' TTACGTATGGCATTTAGTCATTTAATAAGGAAAGAATCAATAAAATAGTTAGTTCCTTCATAATTGCT
 3' AATGCATACCGTAAATCAGTAAAATTATTCCTTTCTTAGTTATTTTATCAATCAAGGGAAGTATTAACGA

6510

BGT27_T18a

BGT27_T18a-pDONR221

L R M A F S H F N K E R I N K I V S S L H N C
 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434

BGT27_T18a-pET32-DEST

5' CTAATTTTAAAGCTATTTTAGATAAAATAACTTTAAGTGATACTTATAATCAACGTATAGCTTACTTTAA
 0 ++++++
 3' GATTAAAATTTTCGATAAAATCTATTTTATTTGAAATTCACATGAAATATTAGTTGCATATCGAATGAAATT

6580

BGT27_T18a

BGT27_T18a-pDONR221

S N F K A I L D K I T L S D T Y N Q R I A Y F K
 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458

5' ACGTCGTCCTATATGATGATAATTGGTTCTTCGATAAAATTTAATATACCCTTCTAGGACCCAGCTTTTCTTG
 0 ++++++
 3' TGCAGCAGATATACTACTATTAACCAAGAAGCTATTTAAATTATATGGGAAGATCCTGGGTCGAAAGAAC

6650

BGT27_T18a

attB2

BGT27_T18a-pDONR221

R R L Y D D N W F F D K F N I P F .
 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476

BamHI

EcoICRI
Sacl

Sall

NotI
EagI

PspXI
XhoI
AvaI
BmeT110I

5' TACAAAGTGGTTGATatcggatccgaattcgagctccgctcgacaagcttgcgccgcactcgagcaccac
 0 ++++++
 3' ATGTTTCACCAACTatagcctaggccttaagctcgaggcagctggtcgaacgccggcggtgagctcggtggtg

6720

attB2

BipI

5' caccaccaccactgagatccggctgctaacaaagcccgaaggaagctgagttggctgctgccaccgctg
 0 ++++++
 3' gtggtggtggtgactctaggccgacgattgtttcgggctttccttcgactcaaccgacgacggtggcgac

6790

T7...r

5' agcaataactagcataacccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaaac
 0 ++++++
 3' tcggtattgatcgtattggggaaccccgagatgtgccagaactccccaaaaaacgactttcctccttg

6860

T7 Terminator

5' tatatccggat
 0 ++++++
 3' atataggccta

6871