

BGT02_T03b-pET32-DEST

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3' accgcttaccctgcgcgggacatcgccgcgtaatttcgcgcccggcacaccaccaatgcgcgtcgcaactgg

5' gctacacttgccagcgcacctagcgcgccgtcctttcgttttctcccttcctttctcgccacgttcgccc 140
o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' cgatgtgaacggtcgcgggatcgcgggcgaggaaagcgaagaagggaaggaaagagcgggtgcaagcggc

5' gctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcga 210
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o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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5' ttgacgttggagtcacgttctttaatagtgactcttgttccaaactggaacaacactcaaccctatct 350
o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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o
o
o
5' cggctctattcttttgatttataagggattttgcggatctcggcctattgggttaaaaaatgagctgattta 420
o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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5' acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg 490
o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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5' tgcgcggaaccctatttgtttattttctaaatacattcaaatatgtatccgctcatgagacaataacc 560
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o
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o
o
o
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o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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o
o
o
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Psil

Ap

Ap

Ap

Ap

0
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0
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0
Ap

910

Scal

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0
3' gtcagtgtcttttcgtagaatgcctaccgtactgtcatctcttaataacgtcacgacggtatgggtactc
0
Ap

980

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0
Ap

1050

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Ap

1120

PstI

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0
Ap

1190

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Ap


1260

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0
Ap

1330

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Ap

1400

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Ap 

1470

BGT02_T03b-pET32-DEST

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 3' tgcgagctaaaaaactacgagcagtcctcccgctcgatacctttttgcggtcgttgcgccggaaaaa
 o
 o
 o Ori

2240

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 o ++++++
 3' tgccaaggaccggaaaaacgaccggaaaaacgagtgtacaagaaaggacgcaataggggactaagacaccta
 o
 o
 o PciI
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 o ++++++
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 o
 o
 o SapI

2310

2380

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 o ++++++
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 o
 o
 o BstZ17I

2450

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 o
 o BstZ17I

2520

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2590

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2660

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2730

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2800

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 o BstZ17I

2870

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2940

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3010

BGT02_T03b-pET32-DEST

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 0
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 0
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3080
 3150
 3220
 3290
 3360
 3430
 3500
 3570
 3640
 3710
 3780

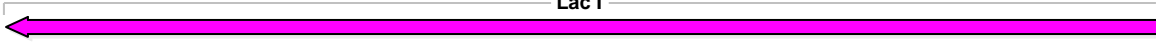
Bpu10I

FspAI

PshAI

Lac I

Lac I



BGT02_T03b-pET32-DEST

5' gattgcccttcaccgacctggccctgagagagtgtgcagcaagcgggtccacgctggtttgccccagcaggcg 3850
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ctaacgggaagtggcggaccgggactctctcaacgctcgttcgccaggtgcgaccaaaccggggctcgtccgc
 Lac I

5' aaaatcctgtttgatgggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccact 3920
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ttttaggacaaactaccaccaattgccgcctatatattgtactcgcagagaagccatagcagcataggggtga
 Lac I

5' accgagatgtccgcaccaacgcgcagcccggactcggtaatggcgcgcatcgcgccagcgcctatctgat 3990
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' tggctctacaggcgtgggttgcgctcgggacctgagccattaccgcgcgtaacgcgggtcgcggtagacta
 Lac I

5' cgttggcaaccagcatcgcagtgggaaacgatgccctcattcagcatttgcattggttggtaaaaccgga 4060
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' gcaaccgttggctcgtagcgtcacccttgctacgggagtaagtcgtaaaccgtaccaacaactttggcct
 Lac I

5' catggcactccagtcgccttcccgttccgctatcggctgaatttgattgcgagtgagatatttatgccag 4130
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' gtaccgtgaggtcagcgggaagggaaggcgatagccgacttaaacactaacgctcactctataaatacggtc
 Lac I

5' ccagccagacgcagacgcgccgagacagaacttaatgggcccgcctaacagcgcgatttgctggtgacca 4200
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ggtcggctcgcgctcgcgcggctctgtcttgaattaccgggcgattgtcgcgctaaacgaccactgggt
 Lac I

5' atgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataactggtgatgggtgt 4270
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 Lac I

5' ctggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctgg 4340
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 3' gaccagtctctgtagttctttattgcggccttgtaaacacgctccgctcgaagggtgcgttaccgtaggacc
 Lac I

5' tcatccagcggatagttaatgatcagcccactgacgcggttgcgcgagaagattgtgcaccgccgctttac 4410
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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 Lac I

HpaI

BssHII

PspOMI
 ApaI

5' aggcttcgacgccgcttcggttctaccatcgacaccaccacgctggcaccagttgatcggcgcgagattt 4480
o ++++++
3' tccgaagctgccggcgaagcaagatggtagctgtgggtggtgcgaccgtgggtcaactagccgcgctctaaa
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5' aatcgcgcgcgacaatttgcgacggcgcgtgcagggccagactggaggtggcaacgccaatcagcaacgac 4550
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o
o
o

Lac I

Lac I

Lac I

BstAPI

SphI

XbaI

T7 Promoter

Lac Operator

Bam

Bam

5' gtttaactttaagaaggagatatacatatgagcgcataaaattattcacctgactgacgacagttttgaca
3' caaattgaaattcttctctatatgtatactcgctattttaataagtggactgactgctgtcaaaactgt

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

5' cggatgtactcaaagcggacggggcgatcctcgtcgattttctgggcagagtgggtgcgggtccgtgcaaaat
3' gcctacatgagtttcgcctgccccgctaggagcagctaaagaccctctcaccacgccaggcaggttcta

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' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaaactgaccgttgcaaaactgaacatc
3' ctagcggggctaagacctacttttagcgactgcttatagtcctcgcttgactggcaacgttttgacttgtag

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

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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

5' gtgaagtggcggcaaccaagtggtgcaactgtctaaaggtcagttgaaagagttcctcgacgctaacct
3' cacttcaccgcccgttggtttcaccacgtgacagatttccagtcaactttctcaaggagctgcgattgga

5530

Trx Tag

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' ggccggttctggttctggccatatgcaccatcatcatcatcattcttctggtctggtgccacgcggttct
3' ccggccaagaccaagaccgggtatacgtggtagtagtagtagtaagaagaccagaccacgggtgccaaga

5600

T...g

His6-tag

Thrombin

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

BGT02_T03b-pET32-DEST

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 3' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagaccatggctgc

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

5670

En...e

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 3' tgctgctggttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

Enterokinase attB1 TEV site
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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' aggcAACGACGGCGACGGGACGCCCGCGGCTGCGGGCGGGAGAGGCGTTTCGGCCCCTGGGCACGGCC
 3' tccgTTGCTGCCGCTGCCCTGCGGGCGCCGACGCCCGCCCTCTCCGCCAAGCCGGGCGACCCGTGCCCG

BGT02_T03b
 BGT02_T03b-pDONR221

5810

G N D G D G T P A A A A R E R R F G P L G T A
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5' CTGGTGATCATCCCGACCTACAACGAGGCGGAGAACATCAAGGCGATCGTCACGCGTGTGCGCGAGGCCG
 3' GACCACTAGTAGGGCTGGATGTTGCTCCGCCTCTTGTAGTTCCGCTAGCAGTGCGCACACGCGCTCCGGC

BGT02_T03b
 BGT02_T03b-pDONR221

5880

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

5' TCCCCGAGGCACACGTGCTCGTGGCCGACGACAACAGCCCCGACGGCACC GGCAAGCTCGCCGACGAACT
 3' AGGGGCTCCGTGTGCACGAGCACC GGCTGCTGTTGTGCGGGGCTGCCGTGGCCGTTCGAGCGGCTGCTTGA

BGT02_T03b
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5950

V P E A H V L V A D D N S P D G T G K L A D E L
 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248

BGT02_T03b-pET32-DEST

5' GGCGGCCGCGGACGACCACGTCCAGGTCATGCACCGCAAGGGCAAGGAAGGGCTGGGCGCCGCTACCTC
 0 ++++++
 3' CCGCCGGCGCCTGCTGGTGCAGGTCCAGTACGTGGCGTTCCCGTTCCTTCCCGACCCGCGGCGGATGGAG

6020

BGT02_T03b

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

5' GCCGGCTTCCGCTGGGGCCTGGAGCACGGCTACGGCGTGCTGATCGAGATGGACGCCGACGGCTCCCACC
 0 ++++++
 3' CGGCCGAAGGCGACCCCGGACCTCGTGCCGATGCCGCACGACTAGCTCTACCTGCGGCTGCCGAGGGTGG

6090

BGT02_T03b

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

5' AGCCCGAGGAACTGCCCGTCTGCTGACCGCCCTCAAGGGCGCCGACCTCGTCCCTCGGCTCGCGCTGGGT
 0 ++++++
 3' TCGGGCTCCTTGACGGGGCAGACGACTGGCGGGAGTTCCCGCGGCTGGAGCAGGAGCCGAGCGCGACCCA

6160

BGT02_T03b

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

5' GCCCGGCGGCCGGGTGGTCAACTGGCCCAAGTCCCGCGAGATCATCTCGCGTGGCGGCAGCATGTACTCG
 0 ++++++
 3' CGGGCCGCGGCCACCAGTTGACCGGGTTCAGGGCGCTCTAGTAGAGCGCACCCGCGTCTGTACATGAGC

6230

BGT02_T03b

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

BspMI

5' AGAATCGCGCTCGACCTGCCGCTGCGCGACATCACCGCGGGCTACCGCGCCTTCCGCCGCGAGACCCTGG
 0 ++++++
 3' TCTTAGCGCGAGCTGGACGGCGACGCGCTGTAGTGGCCGCCGATGGCGCGAAGGCGGCGCTCTGGGACC

6300

BGT02_T03b

BGT02_T03b-pDONR221

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

BGT02_T03b-pET32-DEST

AbsI PasI BseRI

5' AGGGGCTCGGCCTCGAGGAGGTGCCTCCCAGGGGTACTGCTTCCAGGTTCGACCTCGCCCGCCGGGCGAT
3' TCCCCGAGCCGGAGCTCCTCCAGCGGAGGGTCCCCATGACGAAGGTCCAGCTGGAGCGGGCGGCCCGCTA

6370

BGT02_T03b

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E G L G L E E V A S Q G Y C F Q V D L A R R A I
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' CAAGGCCGGCTATCACGTTCGTGGAGGTGCCGATCACGTTCGTGGAGCGGGAAGTTCGGCGACTCCAAGATG
3' GTTCCGGCCGATAGTGCAGCACCTCCACGGCTAGTGCAAGCACCTCGCCCTTGAGCCGCTGAGGTTCTAC

6440

BGT02_T03b

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

5' AGCCGCGACATCCTGGTGGAGGCCCTGTGGCGGGTCACCACGTGGGGCGTGGGGGAGAGGGTCGGCAAGG
3' TCGGCGCTGTAGGACCACCTCCGGGACACCGCCCAGTGGTGCACCCCGCACCCCTCTCCAGCCGTTCC

6510

BGT02_T03b

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

5' TGCTCGGCCCGACAGGCGGTCCGGCACGGCGGCCGACGGGCAGACGGCCTCGAGCGGGACCGACGGGCG
3' ACGAGCCGGCGCTGTCCGCCAGGCCGTGCCGCCGGCTGCCCGTCTGCCGGAGCTCGCCCTGGCTGCCCGC

6580

BGT02_T03b

BGT02_T03b-pDONR221

V L G R D R R S G T A A D G Q T A S S G T D G R
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' GACGGCCTCGAGCGACAGGTTCGACGGACTCCCTCGACGAGTCGGCGAAGGCGTCCGACGCCAAGGCCTAG
3' CTGCCGGAGCTCGCTGTCCAGCTGCCTGAGGGAGCTGCTCAGCCGCTTCCGCAGGCTGCGGTTCCGGATC

6650

StuI AvrII

BGT02_T03b

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T A S S D R S T D S L D E S A K A S D A K A
459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481

BGT02_T03b-pET32-DEST

