

BGH35\_B04b-pET16-DEST

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0 ++++++ | 70  
3' aagaacttctgcttccccgggagcactatgCGGATAAAAATATCCAATTACAGTACTATTATTACCAAAGA  
0  
0 Zral  
0 | AatlI  
0  
5' tagacgtcaggtggcactTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATT  
0 ++++++ | 140  
3' atctgcagtcaccCGTGAAAAGCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAAGATTTATGTA  
0  
5' caaatatgtatccgctcatgagacaataaccctgataaATGCTTCAATAATATGAAAAAGGAAGAGTAT  
0 ++++++ | 210  
3' GTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTACGAAGTATTATAACTTTTTCTTTCTCATA  
0  
0  
5' gagtattcaacatttccgTGTGCGCCCTTATCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTGCTCAC  
0 ++++++ | 280  
3' CTCATAAGTTGTAAGGCACAGCGGGAATAAGGGAAAAAACGCCGTA AAACGGAAGGACAAAACGAGTG  
0  
0 Ap  
0  
5' ccagaaacgctggTgaaAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAAGTGG  
0 ++++++ | 350  
3' GGTCTTTGCGACCCTTTCAATTTCTACGACTTCTAGTCAACCCACGTGCTCACCCAATGTAGCTTGACC  
0  
0 Ap  
0  
5' atctcaacagcggTaaGATCCTTGAGAGTTTTCGCCCCGAAGAAGCTTTTCCAATGATGAGCACTTTTAA  
0 ++++++ | 420  
3' TAGAGTTGTGCGCATTCTAGGAACTCTCAAAGCGGGGCTTCTTGCAAAGGTACTACTCGTGA AAATT  
0  
0 Ap  
0  
5' agttctgctatgtggcgcggTATTATCCCGTGTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATAAC  
0 ++++++ | 490  
3' TCAAGACGATACACCGCGCCATAATAGGGCACA ACTGCGGCCGTTCTCGTTGAGCCAGCGGCGTATGTG  
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0 Ap  
0  
5' tattctcagaatgacttggTtgagtactcaccagtcacagaaaagcatctTACGGATGGCATGACAGTAA  
0 ++++++ | 560  
3' ATAAGAGTCTTACTGAACCAACTCATGAGTGGTCAGTGTCTTTTCGTAGAATGCCTACCGTACTGTCA  
0  
0 Ap  
0  
5' gagaattatgcagtgctgccATAACCATGAGTGATAAACTGCGGCCAACTTACTTCTGACAACGATCGG  
0 ++++++ | 630  
3' CTCTTAATACGTCACGACGGTATTGGTACTCACTATTGTGACGCCGTTGAATGAAGACTGTTGCTAGCC  
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0 Ap  
0  
5' aggaccgaaggagctaacCGCTTTTTGCAACAATGGGGATCATGTA ACTCGCCTTGATCGTTGGGAA  
0 ++++++ | 700  
3' TCCTGGCTTCTCGATTGGCGAAAAACGTTGTACCCCTAGTACATTGAGCGGAAGTAGCAACCCCT  
0  
0 Ap  
0

BGH35\_B04b-pET16-DEST

```

5' cgggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgcagcaatggcaacaacgt
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' ggctcgaacttacttccggtatggtttgctgctgcactgtgggtgctacggacgtcgcttaccgttgttga
  
```

Ap

```

5' tgcgcaaactattaactggcgaactacttacttagcttcccggaacaattaatagactggatggaggc
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' acgcttgataattgaccgcttgatgaatgagatcgaagggccgctgttaattatctgacctacctccg
  
```

Ap

```

5' ggataaagttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatctgga
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' cctatttcaacgtcctggggaagacgagcgggaagccgaccgaccaaataacgactatcttagacct
  
```

Ap

Bsal

```

5' gccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccctcccgtatcgtag
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' cggccactcgcaccagagcgccatagtaacgctcgtgaccccggtctaccattcgggagggcatagcatc
  
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Ap

AhdI


```

5' ttatctacacgacggggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctc
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' aatagatgtgctgcccctcagtcggttgatacctacttgctttatctgcttagcgactctatccacggag
  
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Ap

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5' actgattaagcattggtaactgtcagaccaagtttactcatatatacttttagattgatttaaaacttcat
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' tgactaattcgtgaaccattgacagtctggttcaaataagtagatatatgaaatctaactaaatcttgaagta
  
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Ap

```

5' ttttaatttaaaggatctagggtgaagatcctttttgataatctcatgaccaaataccttaacgtgagt
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' aaaattaaattttcctagatccacttctaggaaaaactattagagtagtggtttagggaattgcactca
  
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```

5' tttcgttccactgagcgtcagaccccgtagaaaagatcaaaggatccttctgagatccttttttctgcg
0 ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3' aaagcaaggtgactcgcagctcggggcatcttttctagtttcttagaagaactctaggaaaaaaagacgc
  
```

Ori

```

5' cgtaatctgctgcttgcaaacaaaaaaaaccaccgctaccagcggtggtttgcttgccggatcaagagcta
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3' gcattagacgacgaacgtttgtttttttggtggcgatggtcgccaccaaaacaaacggcctagttctcgat
  
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Ori

BGH35\_B04b-pET16-DEST

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      Ori
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3' gcatcaatccgggtggagaagtcttgagacatcgtggcgatgtatggagcgagacgattaggacaatgg
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o
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      Ori
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      Ori
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o
      Ori
o
o
o
o
      Pcil
o
o
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o
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3' ataccttttgcggtcgttgcgccggaaaaatgccaaggaccggaaaaacgaccggaacgagtgtacia
o
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3' gaaaggacgcaataggggactaagacacctattggcataatggcggaactcactcgactatggcgagcg
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1400

1470

1540

1610

1680

1750

1820

1890

1960

2030

BGH35\_B04b-pET16-DEST

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+ + + + +  
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Accl  
BstZ171  
Tth111I

5' gcatagttaagccagtatacactccgctatcgctacgtgactgggtcatggctgcgccccgacacccgcc 2170  
+ + + + +  
3' cgtatcaattcggtcatatgtgagggcgatagcgatgcactgaccacgtagcgagggctgtgggcgg  
o  
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5' aacacccgctgacgcgcccctgacgggcttgtctgctcccggcatccgcttacagacaagctgtgaccgctc 2240  
+ + + + +  
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3' ctacgccgcccctggctctcttttagtgagtcccagttacggctcgcaagcaattatgtctacatccacaa  
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+ + + + +  
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o

FspAI

|

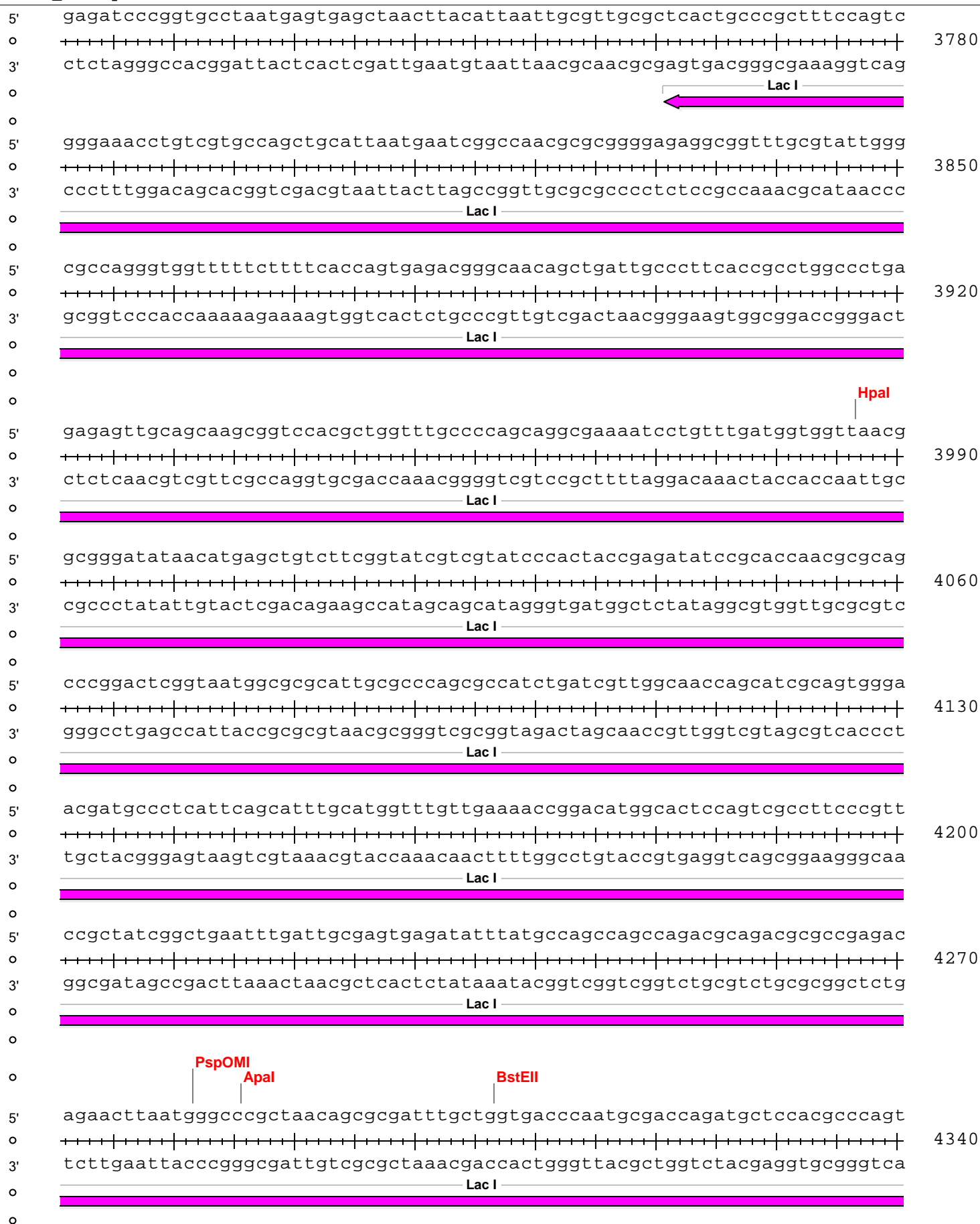
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3' ttacccttccggtaggtcggagcgcagcgttgcggctcgttctgcatcgggtcgcgcagccggcgggtac  
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PshAI

|

o  
5' gcgacgatagtcatgccccgcgcccaccggaaggagctgactgggttgaaggctctcaagggcatcggtc 3710  
o  
3' cgctgctatcagtaggggcccgggtggccttccctcgactgacccaacttccgagagttcccgtagccag  
o

BGH35\_B04b-pET16-DEST





BGH35\_B04b-pET16-DEST

5' agtcccccgccacggggcctgccaccatacccacgcccgaacaagcgctcatgagccccgaagtggcgag  
 0 ++++++  
 3' tcagggggccgggtgccccggacgggtggatgggtgcggtcttggtcgagtagtactcgggcttcaccgctc

5110

SgrAI

5' cccgatcttccccatcggatggtcggcgatataggcgccagcaaccgcacctgtggcgccgggtgatgcc  
 0 ++++++  
 3' gggctagaaggggtagccactacagccgctatatcccgcggtcgttggcgtggacaccgcggccactacgg

5180

BglII

5' ggccacgatgCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGG  
 0 ++++++  
 3' ccggTgctacgcaggccgcAtctcctagctctagagctagggcgctttaaattatgctgagtgatatcccc

5250

T7 Promoter

5' aattgtgagcggataacaattcccctctagaataattttgtttaactttaagaaggagatataccatgg  
 0 ++++++  
 3' ttaacactcgctattgTtaaggggagatctttattaaacaaattgaaattcttcctctatatggTacc

5320

Lac O

M  
1

5' gccatcatcatcatcatcatcatcatcacagcagcggccatatcgaaggTcgtcatatgctcgaATC  
 0 ++++++  
 3' cggtagtagtagtagtagtagtagtagtgTcgtcgccggTatagcttccagcagTatacagagctTAG

5390

BamHI

NdeI

His10-tag

X-factor

G H H H H H H H H S S G H I E G R H M L E S  
 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

5' AACAAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcAGTCCACTGACTATTCAAGGC  
 0 ++++++  
 3' TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagtTccgTCAGGTGACTGATAAGTTCCG

5460

attB1

TEV site

BGH35\_B04b

BGH35\_B04b-pDONR221

T S L Y K K A G S E N L Y F Q G S P L T I Q G  
 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48

5' AAACAATTAATGCTGAACGATCGTCCGTTTCGAATTATAGCCGGCGCCATCCATTATTTTCGCGTTGTTC  
 0 ++++++  
 3' TTTGTTAATTACGACTTGCTAGCAGGCAAAGCTTAATATCGGCCGCGGTAGGTAATAAAAGCGCAACAAG

5530

BGH35\_B04b

BGH35\_B04b-pDONR221

K Q L M L N D R P F R I I A G A I H Y F R V V  
 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71



BGH35\_B04b-pET16-DEST

5' CGGAGTACTGGCGCGACCCGCTGCTTAAATGGAAGGCATGCGGGTTTAATACGGTTGAAACTTACGTGCC  
 0 ++++++  
 3' GCCTCATGACCGCGCTGGCGGACGAATTTAACTTCCGTACGCCCAAATTATGCCAACTTTGAATGCACGG

5600

BGH35\_B04b

BGH35\_B04b-pDONR221

P E Y W R D R L L K L K A C G F N T V E T Y V P  
 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95

5' GTGGAATTTCCATGAGCCGGAGGAAGGCCGCTTTGTATTCGAGGGCATGGCGGATCTGGAGAAGTTCATC  
 0 ++++++  
 3' CACCTTAAAGGTACTCGGCCTCCTTCCGGCGAAACATAAGCTCCCGTACCGCCTAGACCTCTTCAAGTAG

5670

BGH35\_B04b

BGH35\_B04b-pDONR221

W N F H E P E E G R F V F E G M A D L E K F I  
 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118

5' GCGCTTGCCGGCGAACTGGGATTGTACGCGATTGTTCTCAAGCCCGTACATTTGCGCGGAGTGGGAGT  
 0 ++++++  
 3' CGCGAACGGCCGCTTGACCCTAACATGCGCTAACAAAGCAGGTTCTGGGCATGTAAACGCGCCTCACCTCA

5740

BGH35\_B04b

BGH35\_B04b-pDONR221

A L A G E L G L Y A I V R P S P Y I C A E W E  
 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

5' TTGGCGGATTGCCGGCATGGCTGCTCAAAGACCCCGGCATGCGCCTGCGCTGCAGCTATAAGCCGTTCTT  
 0 ++++++  
 3' AACCGCCTAACGGCCGTACCGACGAGTTTCTGGGGCCGTACGCGGACGCGACGTCGATATTCGGCAAGGA

5810

BGH35\_B04b

BGH35\_B04b-pDONR221

F G G L P A W L L K D P G M R L R C S Y K P F L  
 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

5' GGACAAAGCGGATGCTTATTACGACGAACTGATCCCGCGCCTGACCCCGTTCTGTCCACCAAGGGCGGT  
 0 ++++++  
 3' CCTGTTTCGCCTACGAATAATGCTGCTTGACTAGGGCGCGGACTGGGGCAAGGACAGGTGGTTCCCGCCA

5880

BGH35\_B04b

BGH35\_B04b-pDONR221

D K A D A Y Y D E L I P R L T P F L S T K G G  
 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188

BGH35\_B04b-pET16-DEST

5' CCGCTCATCGCGATGCAGATCGAGAACGAGTACGGCAGCTACGGCAACGATAAAACGTATCTCAACTACC  
 0 ++++++  
 3' GGCGAGTAGCGCTACGTCTAGCTCTTGCTCATGCCGTCGATGCCGTTGCTATTTGCATAGAGTTGATGG

5950

BGH35\_B04b

BGH35\_B04b-pDONR221

P L I A M Q I E N E Y G S Y G N D K T Y L N Y  
 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211

5' TGAAGGAAGCACTCGTCAAACGCGGCGTGGATGTACTGCTATTTACCTCTGACGGACCGGAAGATTTTAT  
 0 ++++++  
 3' ACTTCCTTCGTGAGCAGTTTGCGCCGCACCTACATGACGATAAATGGAGACTGCCTGGCCTTCTAAAATA

6020

BGH35\_B04b

BGH35\_B04b-pDONR221

L K E A L V K R G V D V L L F T S D G P E D F M  
 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235

BbvCI

5' GCTGCAAGGCGGCATGGTTGAAGGCGTATGGGAAACGGTTAATTTCCGATCCCGTTCGGCTGAGGCATTC  
 0 ++++++  
 3' CGACGTTCCGCCGTACCAACTTCCGCATACCCTTTGCCAATTAAGCCTAGGGCAAGCCGACTCCGTAAG

6090

BGH35\_B04b

BGH35\_B04b-pDONR221

L Q G G M V E G V W E T V N F G S R S A E A F  
 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258

5' GCCAAGCTGCAGGAATATCAGCCGGATCAGCCGCTTATGTGCATGGAATTTTGGAACGGCTGGTTCGACC  
 0 ++++++  
 3' CGGTTTCGACGTCTTATAGTCGGCCTAGTCGGCGAATACACGTACCTTAAAACCTTGCCGACCAAGCTGG

6160

BGH35\_B04b

BGH35\_B04b-pDONR221

A K L Q E Y Q P D Q P L M C M E F W N G W F D  
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5' ACTGGGGGGAGACTCATCATAACCGCGGCGCCGCGGATGTTGCCCTGGTGCTTGACGAGATGCTGGCAGC  
 0 ++++++  
 3' TGACCCCCCTCTGAGTAGTATGGGCGCCGCGGCGCCTACAACGGGACCACGAACTGCTCTACGACCGTCG

6230

BGH35\_B04b

BGH35\_B04b-pDONR221

H W G E T H H T R G A A D V A L V L D E M L A A  
 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305



SbfI

5' TCCTGTACGCGACGCATTTAACCGGCCCGCGTCCGGCAAGCCGCTCAACCTGCAGGAGGTTTCATGACCG  
3' AGGACATGCGCTGCGTAAATTGGCCGGGCGCAGGCCGTTTCGGCGGAGTTGGACGTCCTCCAAGTACTGGC

6650

BGH35\_B04b

BGH35\_B04b-pDONR221

I L Y A T H L T G P R P A S R L N L Q E V H D R  
422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445

5' CGCGCTTATCTTTATTGACGGCGTGTTCAAAGGCGTTATTGAACGCTCCAACCCGGAGCATGATCTCGTA  
3' GCGCGAATAGAAATAACTGCCGCACAAGTTTCCGCAATAACTTGCAGGTTGGGCCTCGTACTAGAGCAT

6720

BGH35\_B04b

BGH35\_B04b-pDONR221

A L I F I D G V F K G V I E R S N P E H D L V  
446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468

5' TTTGACGTACCGCCCGGCGGCGTGGAGCTGGCCATTCTCGTCGAGAATATGGGACGGATCAATTACGGTC  
3' AAAGTGCATGGCGGGCCCGCCGCACCTCGACCGGTAAGAGCAGCTCTTATACCCTGCCTAGTTAATGCCAG

6790

BGH35\_B04b

BGH35\_B04b-pDONR221

F D V P P G G V E L A I L V E N M G R I N Y G  
469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491

BsiWI

5' CGCACATGAAGGATGTTAAGGGAATTACCGAAGGCGTACGTTTCGGACAGCAGTTCCTGTTTAATTGGAC  
3' GCGTGTACTTCCTACAATTCCTTAATGGCTTCCGCATGCAAAGCCTGTCGTCAAGGACAAATTAACCTG

6860

BGH35\_B04b

BGH35\_B04b-pDONR221

P H M K D V K G I T E G V R F G Q Q F L F N W T  
492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515

5' GGTGCGCCCGTTGCCGCTGGATGACCTGTCCAAACTGCAATTTCTGCTTTAAGCAGTCAACCGTGCCTT  
3' CCACGCGGGCAACGGCGACCTACTGGACAGTTTGGACGTTAAAAGACGAAATTCGTCAGTTGGCACGGAA

6930

BGH35\_B04b

BGH35\_B04b-pDONR221

V R P L P L D D L S K L Q F S A L S S Q P C L  
516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538

BGH35\_B04b-pET16-DEST

5' CAGCCGTCCTTCTATCGGGGCGAGTTCGAAGTGGATGAGCCGGCCGACACGTTTCCTAAGCATGAAAGGGT  
 0  
 3' GTCGGCAGGAAGATAGCCCCGCTCAAGCTTCACCTACTCGGCCGGCTGTGCAAGGATTTCGTACTTTCCA

7000

————— BGH35\_B04b —————

————— BGH35\_B04b-pDONR221 —————

Q P S F Y R G E F E V D E P A D T F L S M K G  
 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561

5' GGACCAAGGGTGTTCCTATATGAACGGCTTTAATCTGGGTCGTTATTGGGAGATTGCGCCTCAAGAAAC  
 0  
 3' CCTGGTTCACCACAACGGATATACTTGCCGAAATTAGACCCAGCAATAACCCTCTAACCGGGAGTTCCTTG

7070

————— BGH35\_B04b —————

————— BGH35\_B04b-pDONR221 —————

W T K G V A Y M N G F N L G R Y W E I A P Q E T  
 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585

5' GTTGACATTCCGGGGCCGCTGCTCCGCACGGGCAAAAACGAGATCATCGTATTCGAGCTGCACGCAGCG  
 0  
 3' CAACATGTAAGGCCCGCGACGAGGCGTGCCCGTTTTTGTCTAGTAGCATAAGCTCGACGTGCGTTCG

7140

————— BGH35\_B04b —————

————— BGH35\_B04b-pDONR221 —————

L Y I P G P L L R T G K N E I I V F E L H A A  
 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608

5' GAGTCCGCAAGCGTTAGCCTGCTGGACTGCCCGGTATTAATAAGCAATAGGACCCAGCTTTCCTGTACA  
 0  
 3' CTCAGGCGTTTCGCAATCGGACGACCTGACGGGCCATAATTTATTTCGTTATCCTGGGTCGAAAGAACATGT

7210

————— BGH35\_B04b ————— ■

attB2

————— BGH35\_B04b-pDONR221 ————— ■

E S A S V S L L D C P V L N K Q .  
 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625

5' AAGTGGTTGATtcgaggatccggctgctaacaaagcccgaaaggaagctgagttggctgctgccaccgct  
 0  
 3' TTCACCAACTAagctcctagggccgacgattgtttcgggctttccttcgactcaaccgacgacggtggcga

7280

attB2

← T... —

5' gagcaataactagcataaaccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaa  
 0  
 3' ctcggtattgatcgtattggggaaccccgagatttgcccagaactccccaaaaaacgactttcctcctt

7350

————— T7 Terminator ————— ■

5' ctatatccggatatcccgcaagaggcccgagctaccggcataaccaagcctatgcctacagcatccagg  
 0  
 3' gatataggcctatagggcggttctccgggccgctcatggccgatttggttcggatcggatgtcgttaggtcc

7420

