

BGT25\_T02a3-pET16-DEST

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 0  
 5' tagacgtcaggtggcacttttcggggaaatgtgcgcggaaccctatTTTgTTtatttttctaaatacatt  
 0 ++++++  
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 0 ++++++  
 3' gtttatacataggcgagtactctgttattgggactatttacgaagtattataactTTTTccttctcata

70  
140  
210



5' gagtattcaacatttccgtgtcgcccttattccctTTTTTgCGGCATTTGccttctgTTTTgctcac  
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280

Ap

5' ccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtggttacatcgaactgg  
 0 ++++++  
 3' ggtctttgCGaccactttcatTTTctacgacttctagTcaaccacgtgctcacccaatgtagcttgacc

350

Ap

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 0 ++++++  
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420

Ap

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490

Ap

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560

Ap

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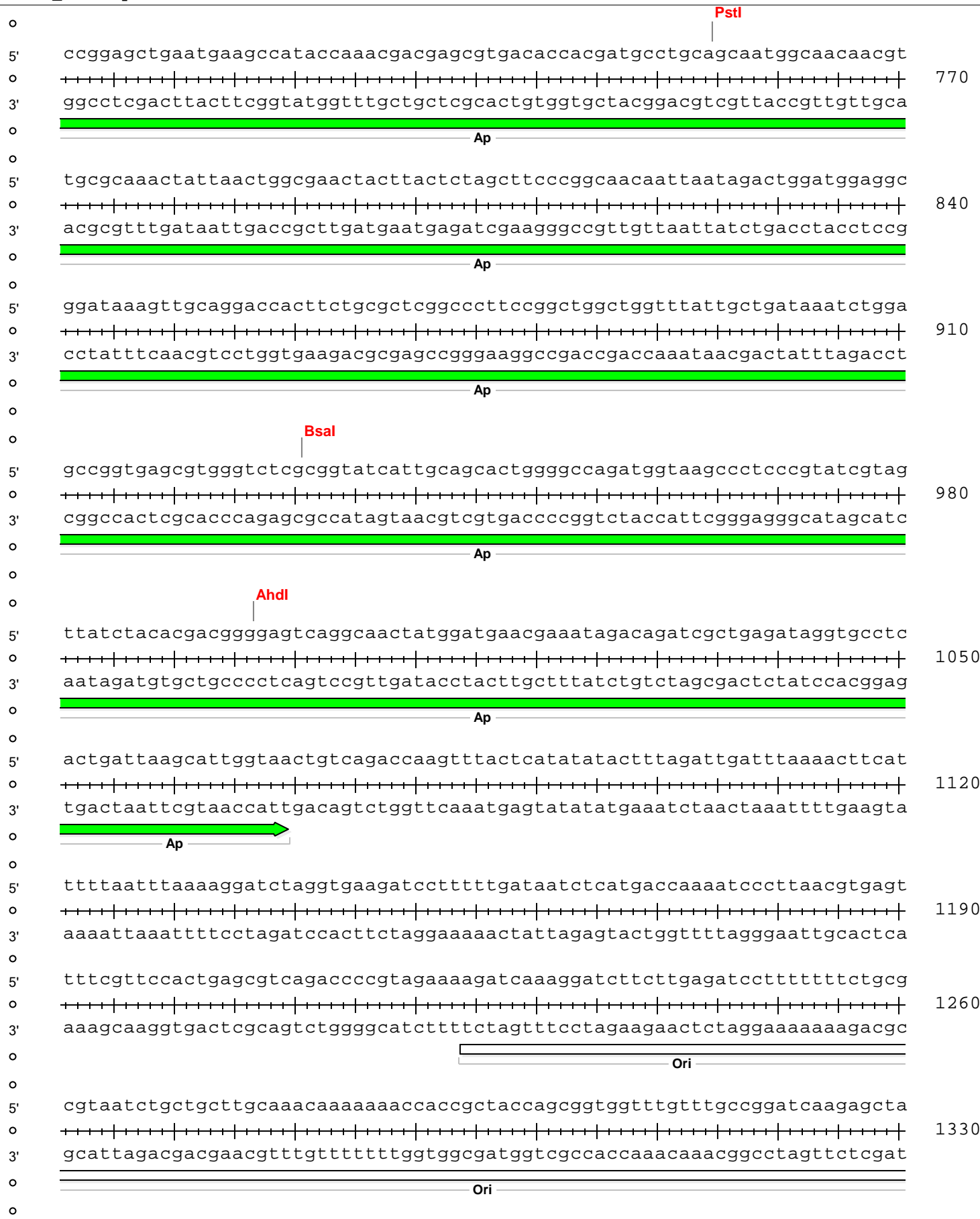
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700

Ap







FspAI

|

o  
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o  
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BspMI

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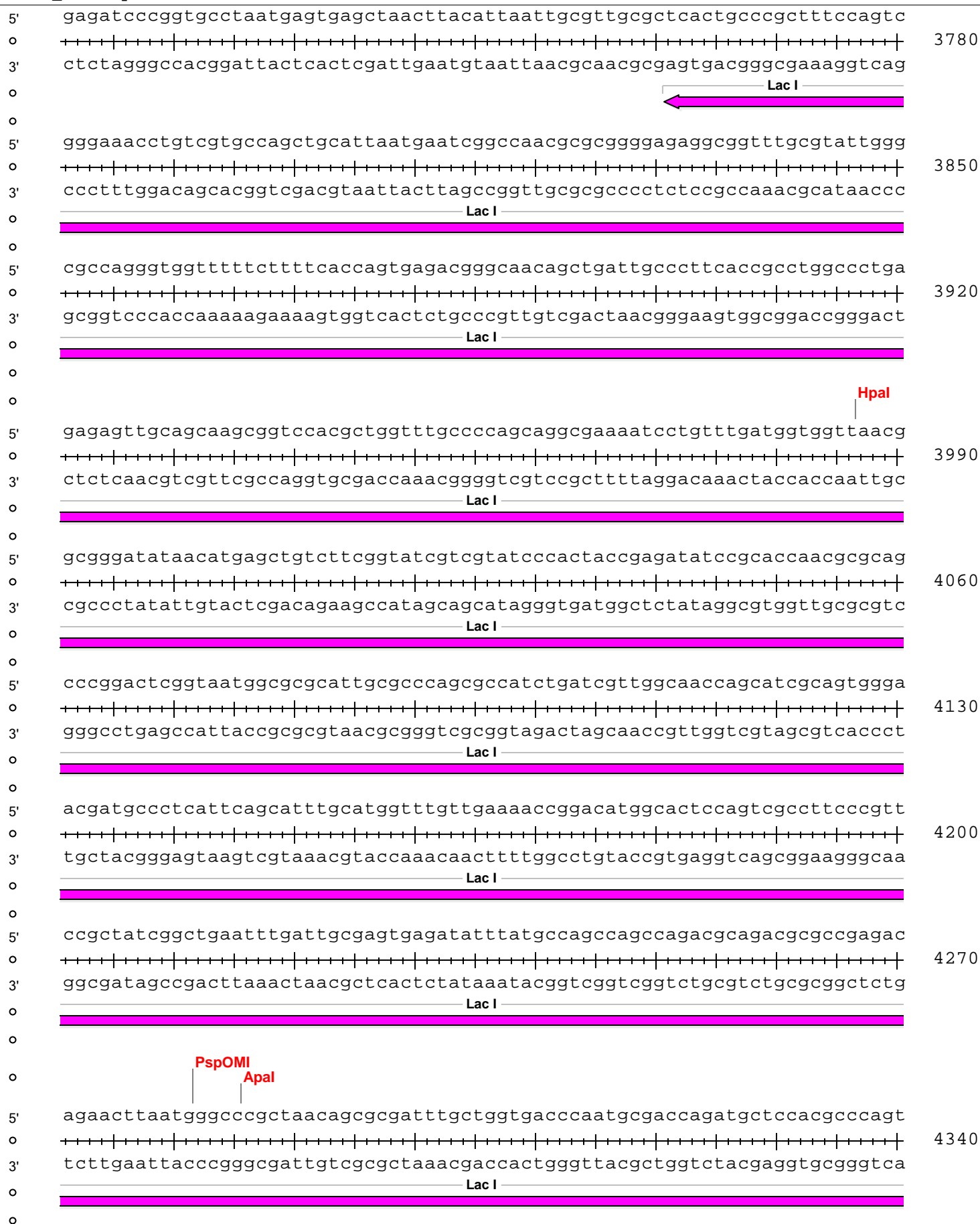
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o  
3' ctttactgggtctcgcgacggccgtggacaggatgctcaacgtactatttcttctgtcagtattcacgc

PshAI

|

o  
5' gcgacgatagtcatgccccgcgccaccggaaggagctgactgggttgaaggctctcaaggcatcggctc 3710  
o  
3' cgctgctatcagtagggggcgcgggtggccttctcgcactgaccaacttcgagagttcccgtagccag

BGT25\_T02a3-pET16-DEST





BGT25\_T02a3-pET16-DEST

5' agtccccggccacggggcctgccaccatacccacgcccgaacaagcgctcatgagccccgaagtggcgag  
 0 ++++++  
 3' tcagggggccgggtgccccggacgggtggatgggtgcggtcttgttcgagtagtactcgggcttcaccgctc

5110

SgrAI

5' cccgatcttccccatcggatgatgctggcgatataggcgccagcaaccgcacctgtggcgccggatgatgcc  
 0 ++++++  
 3' gggctagaaggggtagccactacagccgctatatcccgcggtcgttggcgtggacaccgcggccactacgg

5180

BglII

5' ggccacgatgctcggcgtagaggatcgagatctcgatcccgcgaaattaatacgactcactatagggg  
 0 ++++++  
 3' ccggtgctacgcaggccgcacatctcctagctctagagctagggcgctttaattatgctgagtgatatccc

5250

T7 Promoter

5' aattgtgagcggataacaattcccctctagaataattttgtttaactttaagaaggagatataccatgg  
 0 ++++++  
 3' ttaacactcgctattgttaaggggagatctttattaaacaaattgaaattcttcctctatatggtacc

5320

Lac O

M  
1

5' gccatcatcatcatcatcatcatcatcacagcagcggccatatcgaaggctcgtcatatgctcgaATC  
 0 ++++++  
 3' cggtagtagtagtagtagtagtagtagtagtgtcgtcgccggatagcttccagcagatatacgagctTAG

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' AACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttcaaggcGTCAATGAGCCGCGAAACTT  
 0 ++++++  
 3' TTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagtccgCAGTTACTCGGCGGCTTTGAA

5460

attB1

TEV site

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

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



BGT25\_T02a3-pET16-DEST

5' GGGGCGTTTCGGGACCGAGAGCTTGATCGACCAGCACAGGCTTGTCGGCAGCGGTCGTTGCGGTCGCGCA  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 3' CCCC GCAAGCCCTGGCTCTCGAACTAGCTGGTCGTGTCCGAACAGGCCGTCGCCAGCAACGCCAGCGCGT

**procollagen-lysine 5-dioxygenase**

----- BGT25\_T02a3 -----

----- BGT25\_T02a3-pDONR221 -----

G A F G T E S L I D Q H R L V R Q R S L R S R

49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71

5530

5' TCGAGGGCACGCCGCGCATCCTGGTGACAATCCTCGCAAAGCAGAAAGAGCCGGCACTGCCGCTGTACCT  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 3' AGCTCCCGTGCGGCGCGTAGGACCACTGTTAGGAGCGTTTCGTCTTTCTCGGCCGTGACGGCGACATGGA

**procollagen-lysine 5-dioxygenase**

----- BGT25\_T02a3 -----

----- BGT25\_T02a3-pDONR221 -----

5600

I E G T P R I L V T I L A K Q K E P A L P L Y L

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' CGAGTGCATCGAGGCGCTCGACTACCCCAAGGCGTCGATCGTCCTGTACATCAGGACAAACAACAACACC  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 3' GCTCACGTAGCTCCGCGAGCTGATGGGGTTCGCGCAGCTAGCAGGACATGTAGTCCTGTTTGTGTTGTGG

**procollagen-lysine 5-dioxygenase**

----- BGT25\_T02a3 -----

----- BGT25\_T02a3-pDONR221 -----

5670

E C I E A L D Y P K A S I V L Y I R T N N N T

96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118

5' GACAGGACCCGAGCACATACTGCGCGAATGGGTGGAGCGGTTGGTCACCTCTATGCCGCGGTTGAATTCCG  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 3' CTGTCTGGCTCGTGTATGACGCGCTTACCCACCTCGCGCAACCAGTGGAGATACGGCGCCAACCTTAAGC

**procollagen-lysine 5-dioxygenase**

----- BGT25\_T02a3 -----

----- BGT25\_T02a3-pDONR221 -----

5740

D R T E H I L R E W V E R V G H L Y A A V E F

119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

5' ATGCCTCCAACGTGGCCGATAGAGTCGAGCAGTTTGGCGAACACGAATGGAACGAGACGCGCTTCAGGGT  
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
 3' TACGGAGGTTGCACCGGCTATCTCAGCTCGTCAAACCGCTTGTGCTTACCTTGCTCTGCGCGAAGTCCCA

**procollagen-lysine 5-dioxygenase**

----- BGT25\_T02a3 -----

----- BGT25\_T02a3-pDONR221 -----

5810

D A S N V A D R V E Q F G E H E W N E T R F R V

142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

BGT25\_T02a3-pET16-DEST

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 3' CGAACCGGCTTAGGCGTTGTATTCAAACGCCTTTTGGAGAGCTTGTGTGTCGACGCTAAAGATAAAGCAACGG

5880

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

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

BmgBI

5' GACGTCGATAATTTTCGTGCGACCGGCCACGCTACGCGAGCTTGTGCGCTCGACGTGCCGATCGTCGCTC  
 3' CTGCAGCTATTAAAGCACGCTGGCCGGTGCATGCGCTCGAACAGCGCGAGCTGCACGGCTAGCAGCGAG

5950

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

D V D N F V R P A T L R E L V A L D V P I V A  
 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211

XmaI  
SmaI

5' CCCTGCTTCGCTCCATCTCTCCCGGGCAATACTATTTCGAACTACCACGCCGAAATCGACGCCAACGGCTA  
 3' GGGACGAAGCGAGGTAGAGAGGGCCGTTATGATAAGCTTGATGGTGC GGCTTTAGCTGCGGTTGCCGAT

6020

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

P L L R S I S P G Q Y Y S N Y H A E I D A N G Y  
 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235

CspCI

CspCI'  
PmlI

5' CTATATGCAGTGCATCAATATGGGTGGGTGCTCAACCGCCACGTGCGCGGCATTATCGAGATGCCGCTT  
 3' GATATACGTACGCTAGTTATACCCACCCACGAGTTGCGGGTGCACGCGCCGTAATAGCTCTACGGCGAA

6090

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

Y M Q C D Q Y G W V L N R H V R G I I E M P L  
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BGT25\_T02a3-pET16-DEST

5' GTGCACTGCACCTATCTTGTCCGCGCCGACGTACTGCCCGAACTGACATACGAGGACGCGACATCGCGCT  
 6160  
 3' CACGTGACGTGGATAGAACAGGCGCGGCTGCATGACGGGCTTGACTGTATGCTCCTGCGCTGTAGCGCGA

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

V H C T Y L V R A D V L P E L T Y E D A T S R

259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281

5' ACGAGTACGTGATCTTTGCCGACAGCGCCCGCAAGGCCGGCATCGTCCAGTATATGGATAACCGACAAGT  
 6230  
 3' TGCTCATGCACTAGAAACGGCTGTGCGGGCGTTCCGGCCGTAGCAGGTCATATACCTATTGGCTGTTCA

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

BGT25\_T02a3-pDONR221

Y E Y V I F A D S A R K A G I V Q Y M D N R Q V

282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305

5' CTATGGCTACATCACCTTCGGCGATGGCCAATATTATGTGAGTGACGGGATCGAGCGCGCCCGAGCGCTG  
 6300  
 3' GATACCGATGTAGTGGAAGCCGCTACCGGTTATAATACTCACTGCCCTAGCTCGCGCGGGCTCGCGAC

procollagen-lysine 5-dioxygenase

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

Y G Y I T F G D G Q Y Y V S D G I E R A R A L

306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328

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 6370  
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BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

L H G A G D T P V F T A A T P P G A V P M P D

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5' GACATGTCGAGCCCGCGCAAATTCACCTAATAAACTTGGACAGCAGCGTTCGAGCGCTGGCGCAAATATCA  
 6440  
 3' CTGTACAGCTCGGCGGCGTTTAAGTGGATTATTTGAACCTGTCGTCGCAGCTCGCGACCGCGTTTATAGT

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

R H V E P P Q I H L I N L D S S V E R W R K Y Q

352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375

BGT25\_T02a3-pET16-DEST

SgrDI

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6510

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

D R N P H L A A N T I R V S A V D G A S L D R  
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6580

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

S T L Q A L V D D G V I A E D C G Y L P G A L  
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6650

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

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 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445

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 3' CCTGCTGCAGATGAGCAGCAAGTTGAAAGAACTCCTCAGCTGAGCGTAAGACAGGTACCGAGGTCTCTTG

6720

BGT25\_T02a3

GT25?

BGT25\_T02a3-pDONR221

D D V Y S S F N F L E E S T R I L S M A P E N  
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BseRI

BGT25\_T02a3-pET16-DEST

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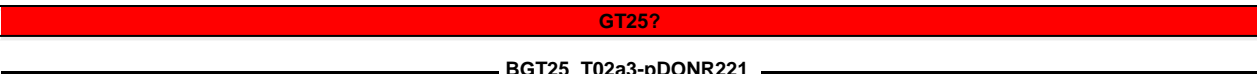
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AjuI'

6790



o

W D M V K W G F N F D P L F L W L N F D F S K

o

469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491

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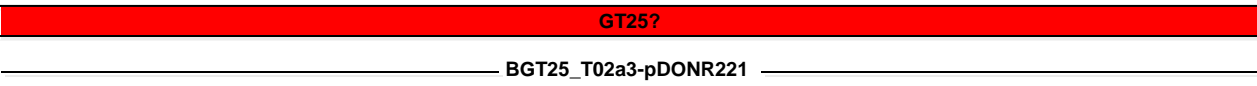
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————— BGT25\_T02a3 —————

AjuI

6860



o

A K L E F Y N R Q H K Q I P A Q F Q S E T Y P R

o

492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515

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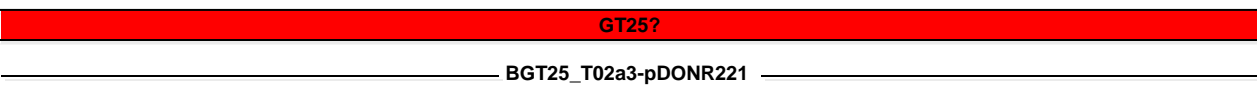
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————— BGT25\_T02a3 —————

SpeI

EcoICRI  
SacI

6930



o

S L I R L V H S F G A M A Y S V T P R G A Q V

o

516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538

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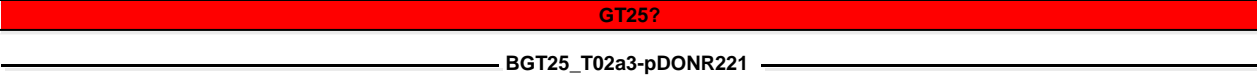
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————— BGT25\_T02a3 —————

AbsI

7000



o

L L E K C L P L R K R L I P F P G T G V V L E

o

539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561

BGT25\_T02a3-pET16-DEST

o

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o

3' TACTCCCGTAGCTGACGCGTTACAGGCGCCACATACTGAGCTACGTACGCAAGCAGACGTACGGCGGTGA

7070

BGT25\_T02a3



BGT25\_T02a3-pDONR221

o

D E G I D C A M S A V Y D S M H A F V C M P P L

o

5' GGTCATTCACGACGACGAGCAGGCATCAGATCGGGAAGTCGCGAATCGGCAGTAGGACCCAGCTTTCTTG

o

3' CCAGTAAGTGCTGCTGCTCGTCCGTAGTCTAGCCCTTCAGCGCTTAGCCGTCATCCTGGGTGAAAGAAC

7140

BGT25\_T02a3

attB2



BGT25\_T02a3-pDONR221

V I H D D E Q A S D R E V A N R Q .

586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603

o

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o

3' ATGTTTCACCAACTAagctcctaggccgacgattgtttcgggctttccttcgactcaaccgacgacggtg

7210

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T7 Terminator

7280

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o

5' cagggtgacggtgccgaggatgacgatgagcgcattgttagatttcatacacggtgccctgactgcgtag

o

3' gtcccactgccacggctcctactgctactcgcgtaacaatctaagatgtgcccacggactgacgcaatc

7350

7420

HindIII

Clal

o

5' caatttaactgtgataaactaccgcattaaagcttatcgatgataagctgtcaaacatgagaa

o

3' gttaaattgacactatttgatggcgtaatttcgaatagctactattcgacagtttgtactcct

7483