



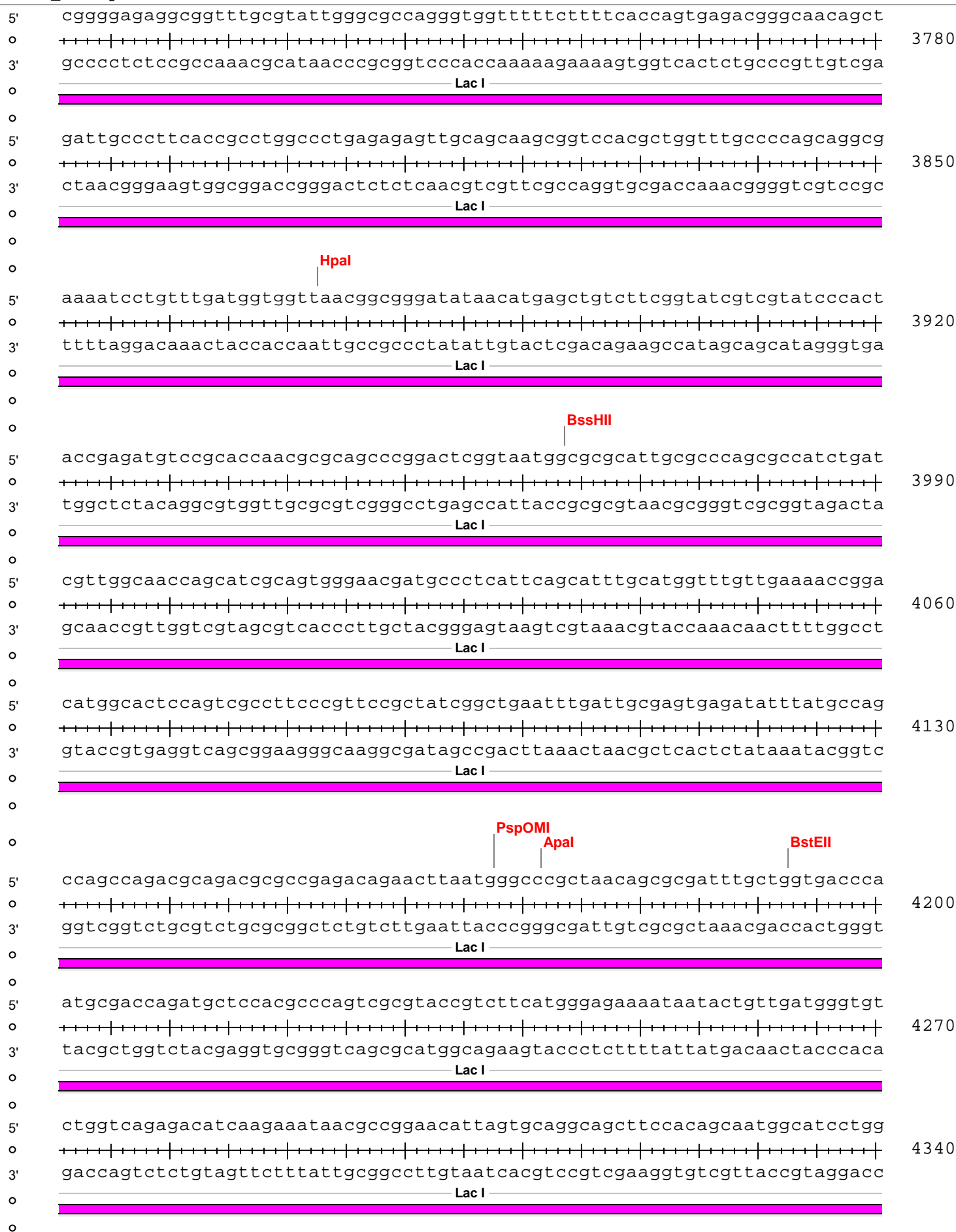








BGH35\_B04c-pET32-DEST



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o  
3' agtaggtcgcctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcgcgaaatg  
o  
Lac I  
o  
5' aggcttcgacgcccgttcggttctaccatcgacaccaccacgctggcaccagttgatcggcgcgagattt 4480  
o  
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o  
Lac I  
o  
5' aatcgccgcgacaatttgcgacggcgcggtgcagggccagactggaggtggcaacgccaatcagcaacgac 4550  
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o  
Lac I  
o  
5' tgtttgcccgccagttggttgccacgcggttgggaatgtaattcagctccgccatcgcccgttccactt 4620  
o  
3' acaaacgggcggtcaacaacacggtgcgccaacccttacattaagtcgaggcggtagcggcgaaaggtgaa  
o  
Lac I  
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BstAPI  
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3' gcgatagtacggatggcgctttccaaaacgcggttaagctaccacaggccctagagctgcgagagggaat  
o  
EcoNI  
o  
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o  
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o  
SphI  
o  
5' gtgcatgcaaggagatggcgcccaacagtccccggccacggggcctgccaccatacccacgcccgaaca 4970  
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o  
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o

o  
 5' accgcacctgtggcgccgggtgatgccggccacgatgCGTCCGGCGTAGAGGATCGAGATCGATCTCGATC  
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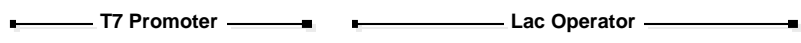
5110

SgrAI

XbaI

o  
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5180

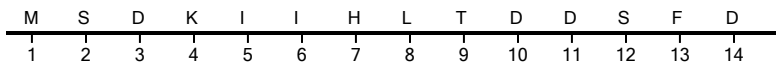


o  
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5250

BarI'

Trx Tag

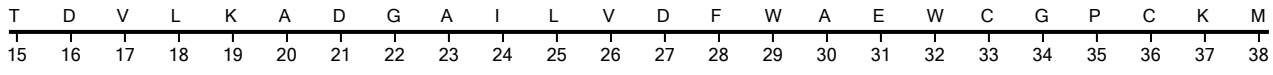


o  
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5320

RsrII

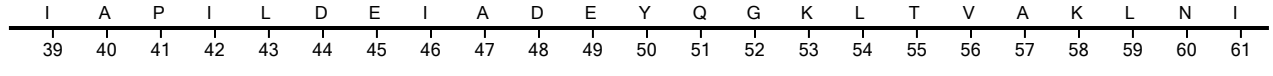
Trx Tag



o  
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5390

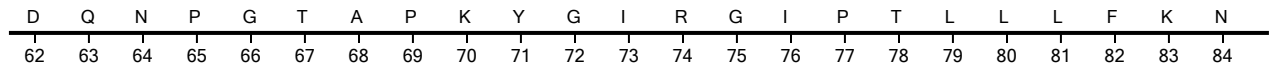
Trx Tag



o  
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 o  
 3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

5460

Trx Tag





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 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

5' ggccggttctgggttctggccatatgcaccatcatcatcattcttctgggtctggtgccacgcggttct  
 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 BglII Acc65I KpnI  
 5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg  
 3' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacctatggctgc  
 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' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca  
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

5740

Enterokinase attB1 TEV site  
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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' aggcTCTCAATCTTCGGAGGGCACATTTGAAGTGGGGAAGAATACTTTCTTGCTGAATGGAGAACCgTTT  
 3' tccgAGAGTTAGAAGCCTCCCGTGTAAACTTCACCCCTTCTTATGAAAGAACGACTTACCTCTTGGCAAA  
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5810

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





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5' GTGCCGAAGAATTGGTAAAAGGTATGAAAGAAATGCTGGACCGCAATATTTCTTTCAGCTTGTATATGAC  
 3' CACGGCTTCTTAACCATTTTCCATACTTTCTTTACGACCTGGCGTTATAAAGAAAGTCGAACATATACTG

6580

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

Spel

5' TCACGGGGGGACTAGTTTCGGGCATTGGGGAGGCGCGAATTTCCCGAACTTTTCACCGACTTGCACATCG  
 3' AGTGCCCCCTGATCAAAGCCCGTAACCCCTCCGCGCTTAAAGGGCTTGAAAAGTGGCTGAACGTGTAGC

6650

BGH35\_B04c

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H G G T S F G H W G G A N F P N F S P T C T S  
 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481

5' TATGATTATGATGCTCCTATCAATGAATCCGGTAAGGTGACTCCTAAATATCTGGAAGTACGGAATCTGC  
 3' ATACTAATACTACGAGGATAGTTACTTAGGCCATTCCACTGAGGATTTATAGACCTTCATGCCTTAGACG

6720

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Y D Y D A P I N E S G K V T P K Y L E V R N L  
 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504

5' TTGGTAACTACCTGCCGGAAGGTGAAACCTTGCCGGAAATTCGGATTCGATACCGACTATAGCTATTCC  
 3' AACCATTGATGGACGGCCTTCCACTTTGGAACGGCCTTTAAGGCCTAAGCTATGGCTGATATCGATAAGG

6790

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L G N Y L P E G E T L P E I P D S I P T I A I P  
 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528

5' TACTATTAATGACTGAAATGGCAGTCTTATTTGATAATCTGCCTCACCCCAAAGAGAGTGAGGATATC  
 3' ATGATAATTTTACTGACTTTACCGTCAGAATAAACTATTAGACGGAGTGGGGTTTCTCTCACTCCTATAG

6860

BGH35\_B04c

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T I K M T E M A V L F D N L P H P K E S E D I  
 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551

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PsrI

5' CGGACAATGGAAGCTTTTGGATCAGGGATGGGGTAGTATTCTGTATCGGACTTCATTATCCGCTTCTGATA  
3' GCCTGTTACCTTCGAAAACCTAGTCCCTACCCCATCATAAGACATAGCCTGAAGTAATAGGCCGAAGACTAT

6930

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R T M E A F D Q G W G S I L Y R T S L S A S D  
552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574

PsrI'

5' AAGAACAGACATTACTGATCACAGAAGCTCATGACTGGGCACAAGTCTTTCTGAATGGTAAGAACTGGC  
3' TTCTTGTCTGTAATGACTAGTGTCTTCGAGTACTGACCCGTGTTTCAGAAAGACTTACCATTCTTTGACCG

7000

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K E Q T L L I T E A H D W A Q V F L N G K K L A  
575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598

5' TACTCTGAGCCGTTTGAAGGGGGAAGGTGTAGTAAAGTTACCACCATTGAAAAGAAGGTGACAGACTGGAT  
3' ATGAGACTCGGCAAACTTCCCCCTTCCACATCATTTC AATGGTGGTAACTTTCTTCCACTGTCTGACCTA

7070

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T L S R L K G E G V V K L P P L K E G D R L D  
599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621

5' ATCCTGGTGGAAAGCAATGGGAAGAATGAAATTCGGCAAAGGTATTTATGACTGGAAAGGTATTACCGAGA  
3' TAGGACCACCTTCGTTACCCTTCTTACTTAAAGCCGTTTCCATAAATACTGACCTTTCATAATGGCTCT

7140

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I L V E A M G R M N F G K G I Y D W K G I T E  
622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644

5' AAGTAGAACTGCAATCTGATAAAGGAGTTGAACTGGTGAAGGACTGGCAGGTATATACTATTCGGGTAGA  
3' TTCATCTTGACGTTAGACTATTTCCCTCAACTTGACCACCTTCCTGACCGTCCATATATGATAAGGCCATCT

7210

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K V E L Q S D K G V E L V K D W Q V Y T I P V D  
645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668

BsmI

5' TTATAGTTTTGCCCGTGATAAGCAGTATAAACAACAGGAGAATGCTGAAAATCAACCGGCTTATTATCGG  
3' AATATCAAACGGGCACTATTCGTCATATTTGTTGTCCTCTTACGACTTTTGTAGTTGGCCGAATAATAGCC

7280

BGH35\_B04c

BGH35\_B04c-pDONR221

Y S F A R D K Q Y K Q Q E N A E N Q P A Y Y R  
669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691

5' TCGACGTTTAACTCAATGAGTTAGGGGATACATTCTTGAATATGATGAACTGGAGTAAAGGTATGGTAT  
3' AGCTGCAAATTGGAGTTACTCAATCCCCTATGTAAGAACTTATACTACTTGACCTCATTTCCATAACCATA

7350

BGH35\_B04c

BGH35\_B04c-pDONR221

S T F N L N E L G D T F L N M M N W S K G M V  
692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714

5' GGGTGAATGGCCATGCGATTGGTCGTTATTGGGAAATCGGACCTCAACAGACCTTATATGTACCGGGCTG  
3' CCCACTTACCGGTACGCTAACCCAGCAATAACCCTTTAGCCTGGAGTTGTCTGGAATATACATGGCCCGAC

7420

BGH35\_B04c

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W V N G H A I G R Y W E I G P Q Q T L Y V P G C  
715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738

5' CTGGTTGAAGAAAGGAGAGAATGAAATCATTATATTGGATATGGCTGGTCCTTCAAAGCTGAAACTGAA  
3' GACCAACTTCTTTCCTCTCTTACTTTAGTAATATAACCTATAACCGACCAGGAAGTTTTCGACTTTGACTT

7490

BGH35\_B04c

BGH35\_B04c-pDONR221

W L K K G E N E I I I L D M A G P S K A E T E  
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5' GGTCTGCGTCAACCAATTCTGGATGTACAACGTGGTAACGGAGCTTATGCTCATCGTAAGATGGGTGAGA  
3' CCAGACGCAGTTGGTTAAGACCTACATGTTGCACCATGTCCTCGAATACGAGTAGCATTCTACCCACTCT

7560

BGH35\_B04c

BGH35\_B04c-pDONR221

G L R Q P I L D V Q R G N G A Y A H R K M G E  
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5' ACTTGACTTAACAAATGAAACTCCTGTTTATCAAGGGATATTTAAGTCAGGCAATGGTTGGCAACATGT  
 0 ++++++  
 3' TGAACCTGAATTGTTTACTTTGAGGACAAATAGTTCCTATAAATTCAGTCCGTTACCAACCGTTGTACA

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————— BGH35\_B04c-pDONR221 —————

N L D L T N E T P V Y Q G I F K S G N G W Q H V  
 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808

7630

5' GTAGGACCCAGCTTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgtcgacaagcttgc  
 0 ++++++  
 3' CATCCTGGGTTCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacg

■ attB2

— BGH35\_B...DONR221 —■

809

7700

5' ggccgcactcgagcaccaccaccaccactgagatccggctgctaacaaagcccgaaaggaagctgag  
 0 ++++++  
 3' ccggcgtgagctcgtggtggtggtggtgactctaggccgacgattgtttcgggctttccttcgactc

NotI PspXI XhoI Aval BmeT110I

BlnI

————— T7 Terminator —————

7770

7840

5' ttttgctgaaaggaggaactatatccggat  
 0 ++++++  
 3' aaaacgactttcctccttgatataggccta

— T... —■

7870