

BGH18_B05b-pET32-DEST

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o
o
Ap
840

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o
o
Ap
Scal
910

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980

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o
o
o
Ap
PvuI
1050

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Ap
1120

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PstI
1190

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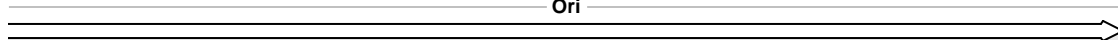
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Ap
Bsal
1330

BGH18_B05b-pET32-DEST

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 Ori

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 2170
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 Ori

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 2240
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 Ori



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 2310
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o

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o
    FspAI
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    |
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o

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 Lac I ←

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 Lac I

HpaI

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PspOMI Apal BstEII

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 Lac I

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 Lac I

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XbaI

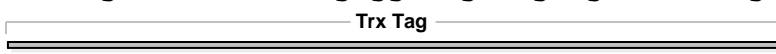
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5180



o
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5250



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

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5320

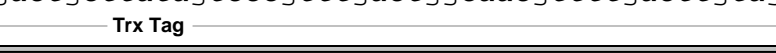
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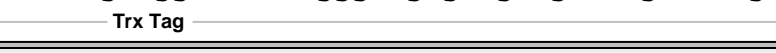
5390



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5460



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BGH18_B05b-pET32-DEST

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5530

Trx Tag

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MscI

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5600

-T...g
 A G S G S G H M H H H H H S S G L V P R G S
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His6-tag

Thrombin

BglIII

Acc65I

KpnI

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5670

En...e

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 0 ++++++
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5740

Enterokinase attB1 BGH18_B05b-pDONR221

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5810

BGH18_B05b-pDONR221

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 0 ++++++
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5880

BGH18_B05b-pDONR221

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BGH18_B05b-pET32-DEST

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 _____ **BGH18_B05b-pDONR221** _____

5950

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 _____ **BGH18_B05b-pDONR221** _____

6020

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 3' TGTTTTAGAATAGGACCGAGTCCATGTTGAACCCTTCTAACACTATTTATGCGTGCGACGCCTTTAAAGC
 _____ **BGH18_B05b-pDONR221** _____

6090

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 _____ **BGH18_B05b-pDONR221** _____

6160

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 _____ **BGH18_B05b-pDONR221** _____

6230

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 _____ **BGH18_B05b-pDONR221** _____

6300

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BGH18_B05b-pET32-DEST

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6370

BGH18_B05b-pDONR221

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6440

BGH18_B05b-pDONR221

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6510

BGH18_B05b-pDONR221

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6580

BGH18_B05b-pDONR221

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SnaBI

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6650

BGH18_B05b-pDONR221

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6720

BGH18_B05b-pDONR221

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BGH18_B05b-pET32-DEST

SexAI Agel

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 6790
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————— BGH18_B05b-pDONR221 —————

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5' ATACGATTATGGTGATTTAGCGGCTAATTATGTTAATAAAAATGGTTTTGTTTCGCGGATGGAACGATATA
 6860
 3' TATGCTAATAACCACTAAATCGCCGATTAATAACAATTATTTTTACCAAACAAGCGCCTACCTTGCTATAT

————— BGH18_B05b-pDONR221 —————

Y D Y G D L A A N Y V N K N G F V R G W N 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

5' GCGAAAGTACCTTATTTATATAATGCGACTACAGGTACATTTATTAGCTACGATGATAATGAATCTATGA
 6930
 3' CGCTTTCATGGAATAAATATATTACGCTGATGTCCATGTAATAAATCGATGCTACTATTACTTAGATACT

————— BGH18_B05b-pDONR221 —————

A K V P Y L Y N A T T G T F I S Y D D N E S M

552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574

5' AATATAAAACAGACTATATAAAAACAAAAGTTTAAAGTGGCGCAATGTTTTGGGAGCTTAGCGGAGATTG
 7000
 3' TTATATTTTGTCTGATATATTTTGTTTTCCAAATTCACCGGTTACAAAACCCTCGAATCGCCTCTAAC

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K Y K T D Y I K T K G L S G A M F W E L S G D C

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' CCGTACAAGTCCAAAATATAGTTGTAGTGGACCAAATTACTTGATACGTTAGTAAAAGAATTA TAGGT
 7070
 3' GGCATGTTTCAGGTTTTATATCAACATCACCTGGTTTTAATGAACTATGCAATCATTTTCTTAATGATCCA

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R T S P K Y S C S G P K L L D T L V K E L L G

599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621

5' GGACCTATTACTCAAAAAGATACTGAGCCACCAACAAACGTAAAAAATATTGTTGTTACAAATAAAAATT
 7140
 3' CCTGGATAATGAGTTTTTCTATGACTCGGTGGTTGTTGCATTTTTTATAACAACAATGTTTATTTTTAA

————— BGH18_B05b-pDONR221 —————

G P I T Q K D T E P P T N V K N I V V T N K N

622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644

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BsmI

5' CAAGCTCAGTTCAATTAAACTGGAATGCATCTACTGATAACGTAGGAGTTACGGAATATGAAATTACTGC
 3' GTTCGAGTCAAGTTAATTTGACCTTACGTAGATGACTATTGCATCCTCAATGCCTTATACTTTAATGACG

7210

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

5' TGGGGAAGAGAAATGGAGTACAACAACAAATAACATTACAATTA AAAACTTAAAATCTAATACAGAATAC
 3' ACCCCTTCTCTTTACCTCATGTTGTTGTTTATTGTAATGTTAATTTTGAATTTTAGATTATGTCTTATG

7280

BGH18_B05b-pDONR221

G E E K W S T T T N N I T I K N L K S N T E Y
 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691

5' AAATTTTCAATAATAGCAAAAGATGCCGCTGGGAATAAATCACAGCCTACTTCTATTATTGTAAAAACAG
 3' TTTAAAAGTTATTATCGTTTTCTACGGCGACCCTTATTAGTGTTCGGATGAAGATAATAACATTTTTTGTC

7350

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K F S I I A K D A A G N K S Q P T S I I V K T
 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714

5' ACGATGCTTAGGACCCAGCTTTCTTGTTACAAAGTGGTTGATatcggatccgaattcgagctccgtcgaca
 3' TGCTACGAATCCTGGGTCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgt

7420

BamHI EcoRI EcoRI SacI Sall

attB2

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D D A
 715 716 717 718

5' agcttgcgccgcactcgagcaccaccaccaccactgagatccggctgctaacaaagcccgaaagga
 3' tcgaacgccggcggtgagctcgtggtggtggtggtggtgactctaggccgacgattgtttcgggctttcct

7490

5' agctgagttggctgctgccaccgctgagcaataactagcataacccttggggcctctaaacgggtcttg
 3' tcgactcaaccgacgacgggtggcgactcgttattgatcgtattggggaaccccgagatttgcccagaac

7560

T7 Terminator

HindIII NotI EagI PspXI XhoI Aval BmeT110I

