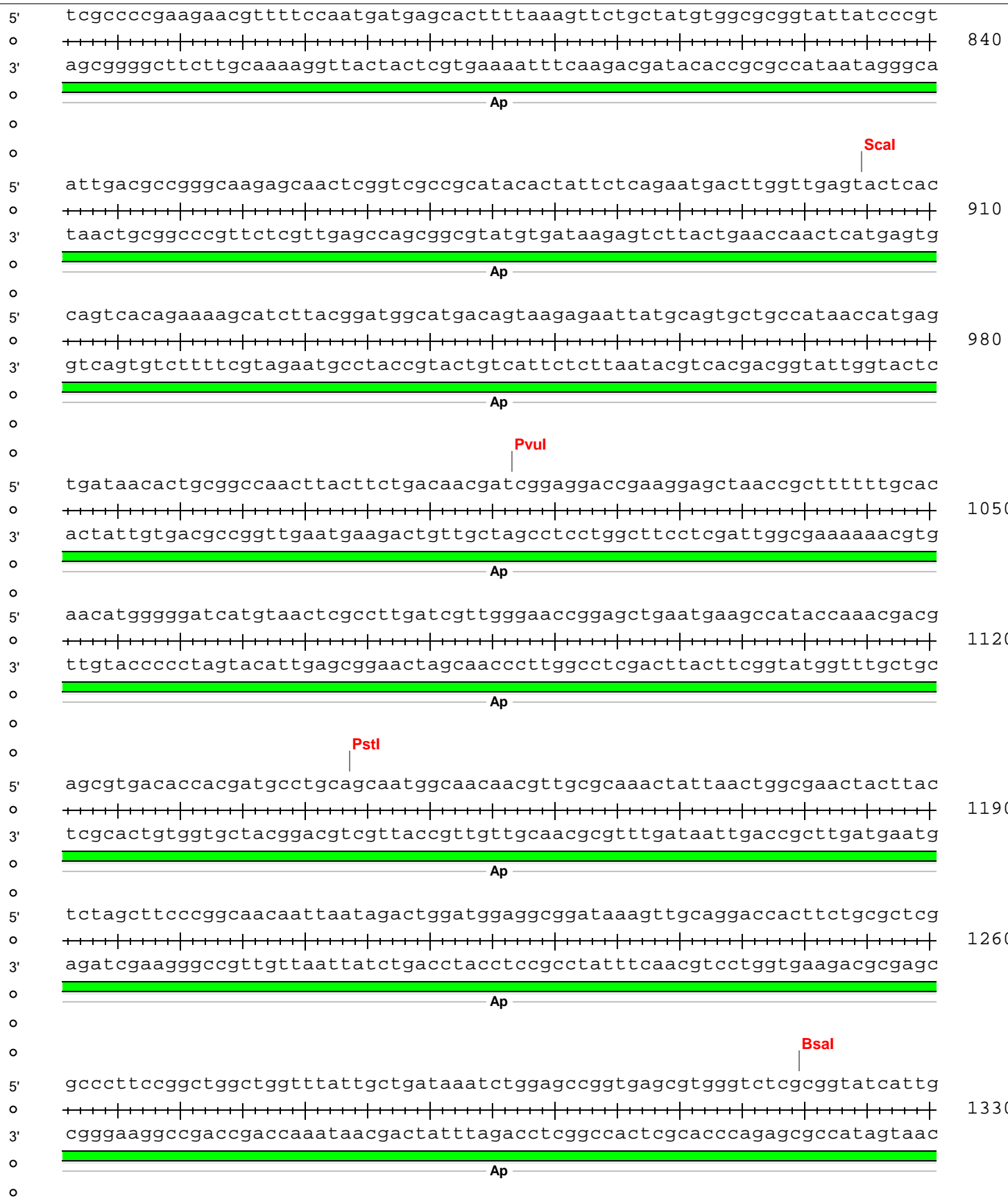


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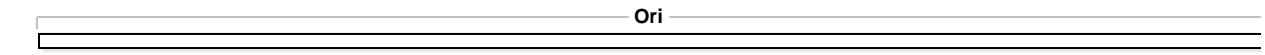
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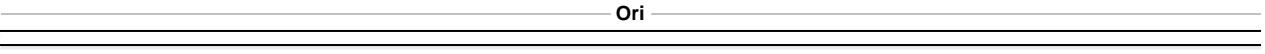
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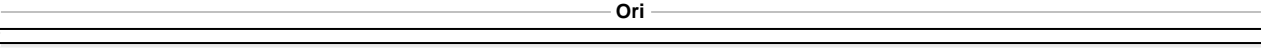
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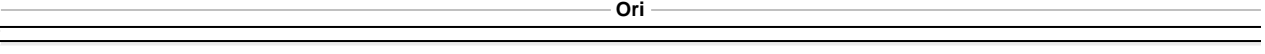
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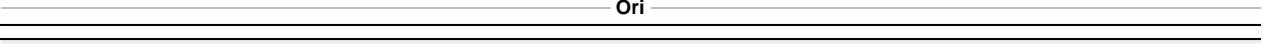
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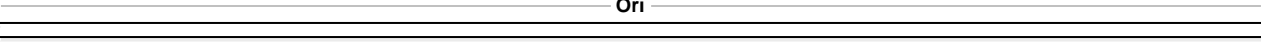
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2030



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 Lac I
 3780

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 Lac I
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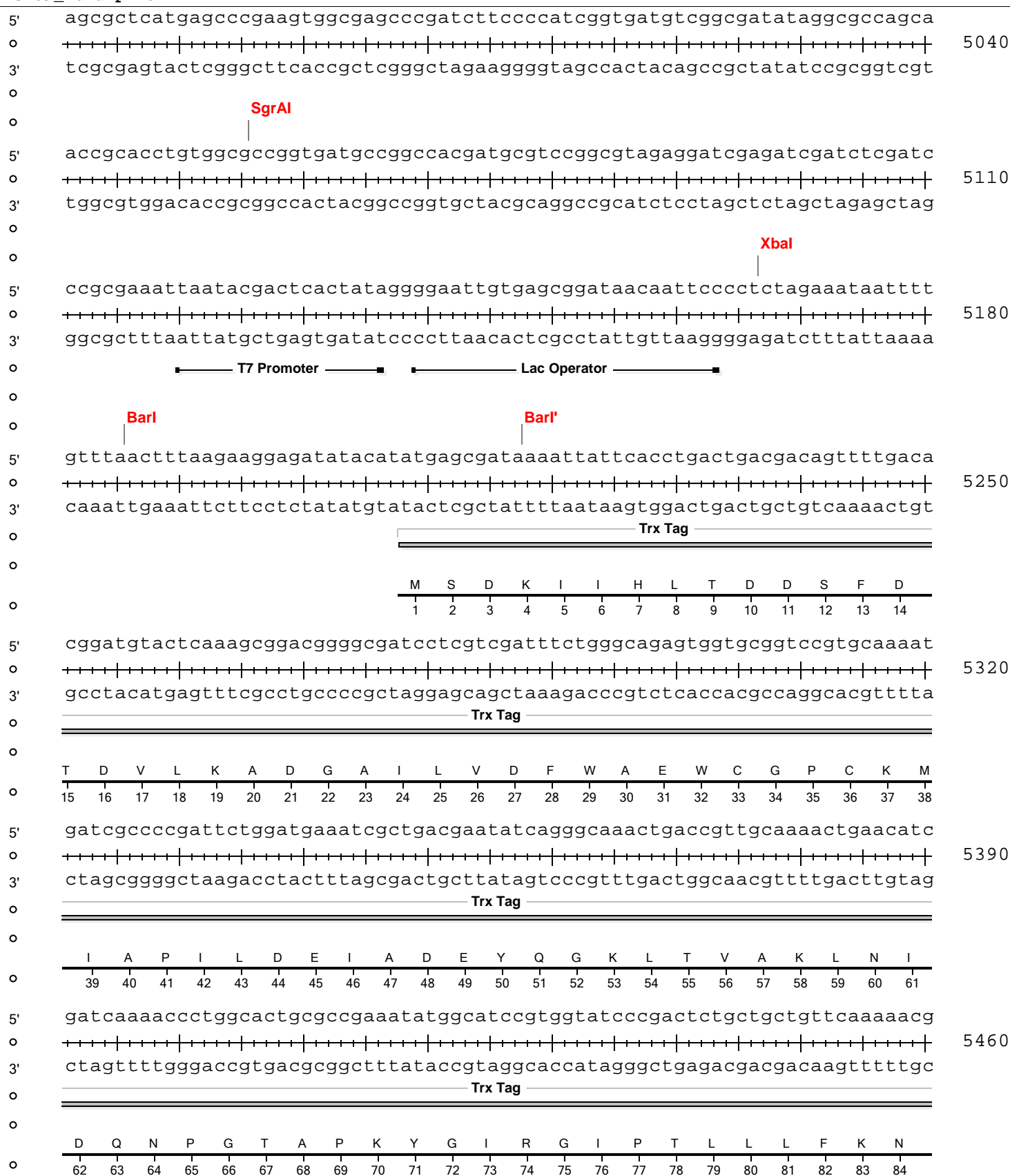
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3' cacttcaccgcgcttggtttcacccacgtgacagatttcagtcacctttctcaaggagctgcgattgga

Trx Tag

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MscI
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+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' ccggccaagaccaagaccggtatagctggtagtagtagtaagaagaccagaccacggtgcgccaaga

-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

BstBI
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En...e
G M K E T A A A K F E R Q H M D S P D L G T D
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+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' tgctgctggtccggtaccgctaTAGTTGTCAAACATGTTTTTTCGTCCGAgacttttgaaacatgaaagt

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+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' tccgGTCGACCTGTTCCGGCGATGCCTCCGAAAACTTTTCCCATCATTTCCCTGTCCCAGATGGCGTAAA

T...

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G Q L D K A A T E A F L K R V V K D R A T A F
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+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' TGGACGCTTATGGACGGACGCCTATTGCCTTTCCTACATAAACTTTATCTTTCATTGCCGTCGGCATAAT

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5950

————— BGH89_B01a —————

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M L R G N N G V S V A S A L N Y Y L R N Y C N S
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5' AATCATTACCTGGAACGGCACCAACCTGCACCTGCCGGTGTATTGCCGGTTGTGAAAGAGAAAGAGCAT
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6020

————— BGH89_B01a —————

————— BGH89_B01a-pDONR221 —————

I I T W N G T N L H L P A V L P V V K E K E H
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 0
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6090

————— BGH89_B01a —————

————— BGH89_B01a-pDONR221 —————

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6160

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6230

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6300

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6370

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 3' CGGTTATAATGGTTCGAAATGGCCTGTGCATGGCGGTCGAAATTTCTATTTATAGGCTTACTCTAGCAT

6440

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6510

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6580

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6650

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 0 ++++++
 3' AGATACCGTCGACGACTAGGCTTTTCGGCACACCCATTACGTCCCTACCTACAAGGTAATATTACGCTCAA

6720

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 0 ++++++
 3' TGACCGTAGTTCGGTGTCTAGTCTCGGAACAATTTACGTACGGACTACTAGTATACTAGCATGACCTAGA

6790

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BGH89_B01a-pDONR221

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 0 ++++++
 3' CATATCACTTTTCAGTAGGACTTACCTCCTTATGTGTCCGAATAATGCCGTTTGGTACCTAGACCTTATAC

6860

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5' CTGCACAACCTTTGGTGGTAATACCGGTATGTGGGGTGGAAATGGATGCCGCTGCACACGATCCGGCTACTG
 0 ++++++
 3' GACGTGTTGAAACCACCATTATGGCCATACACCCACCTTACCTACGGCGACGTGTGCTAGGCCGATGAC

6930

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BGH89_B01a-pDONR221

L H N F G G N T G M W G G M D A A A H D P A T
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A L Y Q L M I D N V W R D Q P I N V D T W L Q
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3' AGCATAACGTTTTGTTGCTATGCCACGTCTCTTACTCCGTCATTTGTTCCGTACCGTCTAGGACATAGTAT

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S Y A K Q R Y G A E N E A V N K A W Q I L Y H
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Ascl

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5' CCGTGTATATTGGCGGTCCGACAGAAGGCGCGCCGGAATCGATCATTTGTTGCAAGACCAACACTGGATAT
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0
3' GGCACATATAACCGCCAGGCTGTCTTCCGCGCGGCCCTTAGCTAGTAACAACGTTCTGGTTGTGACCTATA

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T V Y I G G P T E G A P E S I I V A R P T L D I
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5' CGCAGCAGAGAGAGTAAAGACCAAACCTTGAATATGATCCTGCCAAAGTAGTACCGGCATGGGATCTCTTC
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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3' GCGTCGTCTCTCTCATTCTGGTTTGAACCTTATACTAGGACGGTTTCATCATGGCCGTACCCTAGAGAAG

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A A E R V K T K L E Y D P A K V V P A W D L F
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 7350
 3' TAGTTACGTCGGCGTGTCTGACTTCGGTTGTCTTCCTAAATTCATACTGGACCATCTGGACTGGGCAGTCC

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

5' TGCTTGGTAACTATGCCAGTCCATTACAACAGCGTGTAGCGACTGCTTATCGCAATAAAGATCTGGCGGC
 7420
 3' ACGAACCATTGATACGGTCAGGTAATGTTGTCTGCACATCGCTGACGAATAGCGTTATTTCTAGACCGCCG

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V L G N Y A S P L Q Q R V A T A Y R N K D L A A
 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' ATTTAAACAATACAGCACACAATTCATCGGACTACTGGATGACATGGATATGCTGCTGGGGACTCAGGAA
 7490
 3' TAAATTTGTTATGTCGTGTGTTAAGTAGCCTGATGACCTACTGTACCTATACGACGACCCCTGAGTCCTT

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F K Q Y S T Q F I G L L D D M D M L L G T Q E
 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761

5' GGATTTCTATTGGGCAAATGGGTAAGCGATGCGCGCAGTAATGGTATTACGCCGGCAGAGCAGGACCTGT
 7560
 3' CCTAAAGATAACCCGTTTACCCATTTCGCTACGCGCGTCATTACCATAATGCGGCCGTCTCGTCCTGGACA

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G F L L G K W V S D A R S N G I T P A E Q D L
 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784

5' ATGAGTTCAATGCAAAGACCTGGTGACCTTGTGGGGGGATAAGGATAGTCCGGTACACGAATATTCAAA
 7630
 3' TACTCAAGTTACGTTTTTCTGGACCACTGGAACACCCCTATTCCCTATCAGGCCATGTGCTTATAAGTTT

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

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5' CCGTCAGTGGAAACGGGCTGATTAAAGGCTTTTATAAGCCTCGTTGGCAGCAGTTCTTCACTTTGCTGGAG
 0 ++++++
 3' GGCAGTCACCTTGCCCGACTAATTTCCGAAAATATTCGGAGCAACCGTCGTCAAGAAGTGAAACGACCTC

7700

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BGH89_B01a-pDONR221

R Q W N G L I K G F Y K P R W Q Q F F T L L E
 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831

5' TCCAGCCTCAAAAAGGGCGAAACAGCCGATTTAAAGGCATTTGAAGAGCAGGTGAAGGCATTTGAGTGGGA
 0 ++++++
 3' AGGTCGGAGTTTTTCCCGCTTTGTTCGGCTAAATTTCCGTAAACTTCTCGTCCACTTCCGTAAACTCACCT

7770

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BGH89_B01a-pDONR221

S S L K K G E T A D L K A F E E Q V K A F E W
 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854

5' AATGGGCGAACGGGCATGATAAATATGCCGTAAAGCCACAGGGAGACGCCGTGAAAGCTGCCGTTCAATT
 0 ++++++
 3' TTACCCGCTTGCCCGTACTATTTATACGGCATTTCGGTGTCCCTCTGCGGCACTTTCGACGGCAAGTTAA

7840

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K W A N G H D K Y A V K P Q G D A V K A A V Q L
 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878

5' ACACAAGAAATATCGTAAGATGATGTAGGACCCAGCTTTCTTGTACAAAGTGGTTGATatcggatccgaa
 0 ++++++
 3' TGTGTTCTTTATAGCATTCTACTACATCTGGGTTCGAAAGAACATGTTTCACCAACTAtagcctaggctt

7910

BamHI EcoRI

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attB2

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H K K Y R K M M .
 879 880 881 882 883 884 885 886 887

5' ttcgagctccgctcgacaagcttgcggccgcactcgagcaccaccaccaccactgagatccggctgct
 0 ++++++
 3' aagctcgaggcagctgttcgaacgccggcgtgagctcgtggtggtggtggtgactctaggccgacga

7980

EcoICRI SacI Sall

NotI EagI

PspXI XhoI Aval BmeT110I

5' aacaaagcccgaaggaagctgagttggctgctgccaccgctgagcaataactagcataacccttgggg
 0 ++++++
 3' ttgtttcgggctttccttcgactcaaccgacgacggtggcgactcgttattgatcgtattggggaacccc

8050

BlnI

T7 Terminator

BGH89_B01a-pET32-DEST

5' cctcctaaacgggtcttgaggggttttttgctgaaaggaggaactatatccggat
o ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++
3' ggagatttgcccagaactccccaaaaaacgactttcctccttgatataggccta
o _____ T7 Terminator _____
o

8104