

o
5' tcatccagcggatagttaatgatcagcccactgacgcggtgcgcgagaagattgtgcaccgcccgtttac 4410
o
3' agtaggtcgcctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcgggcaaatg
o
Lac I
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5' aggcttcgacgcccgttcggttctaccatcgacaccaccacgctggcaccagttgatcggcgcgagattt 4480
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Lac I
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3' ttagcggcgctgttaaagcgtgcccgcgacgctcccggctgacctccaccggtgcggttagtcggtgctg
o
Lac I
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5' tgtttgcccgcagttggttgccacgcggttgggaatgtaattcagctccgccatcgcccgttccactt 4620
o
3' acaaacgggcggtcaacaacacggtgcgccaacccttacattaagtcgaggcggtagcggcgaaggtgaa
o
Lac I
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5' tttcccgcggttttcgagaaacgtggctggcctgggtcaccacgcgggaaacggtctgataagagacacc 4690
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3' aaagggcgaaaagcgtctttgcaccgaccggaccaagtgggtgcgccctttgccagactattctctgtgg
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3' cacgtacgttcctctaccgcgggttgctcagggggccggtgccccggacggtggtatgggtgcggtttgt
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5' agcgtcatgagcccgaagtggcgagcccgatcttccccatcgggtgatgtcggcgatatagggcggcagca 5040
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3' tcgagtagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt
o
SgrAI
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5' accgcacctgtggcgccggtgatgccggccacgatgcgtccggcgtagaggatcgagatcgatctcgatc 5110
o
3' tggcgtggacaccgcccactacggccggtgctacgcagggccgcatctcctagctctagctagagctag
o

BGT03_T01c-pET32-DEST

5' ggccggttctggttctggccatatgcaccatcatcatcattcttctggtctggtgccacgcggttct
 3' cccggccaagaccaagaccggtatacgtggtagtagtagtagtaagaagaccagaccacggtgcgccaaga

MscI

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

5600

5' ggtatgaaagaaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg
 3' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacctatggctgc

BglII

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

132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154

5670

5' acgacgacaaggccatggcgcgatATCAACAAGTTTGTACAAAAAGCAGGCTctgaaaacttgtactttca
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

NcoI

Enterokinase **attB1** **TEV site**

BGT03_T01c-pDONR221

5740

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' aggcGTAAAAGATTTATTAACCCCGATTATATCTTCGAGTCCAGCTGGGAAGTATGTAATAAAGTGGGA
 3' tccgCATTTTCTAAATAATTGGGGGCTAATATAGAAGCTCAGGTCGACCCTTCATACATTATTTACCCCT

BGT03_T01c

BGT03_T01c-pDONR221

5810

G V K D L L T P D Y I F E S S W E V C N K V G

179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

5' GGGATATACACCGTATTGTGCGACACGGCGAATACATTGCAGGAAAAATTCCGCGACAGAATTTTTTTCA
 3' CCCTATATGTGGCATAACAGCTGTGCCCGCTTATGTAACGTCCTTTTTAAGGCGCTGTCTTAAAAAAGT

BGT03_T01c

BGT03_T01c-pDONR221

5880

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

202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224

BGT03_T01c-pET32-DEST

5' AAGGAGATCCGTAAGCATGCGGCAGATGTTGCAGAGCAGGCATTGTGGAAACACTTCATTCAATATTATT
 0 ++++++
 3' TTCTCTAGGCATTCGTACGCCGTCTACAACGTCTCGTCCGTAACACCTTTGTGAAGTAAGTTATAATAA

7350

BGT03_T01c

BGT03_T01c-pDONR221

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

5' ACGAGGCTTATGATATTGCCCTTGCGCAATGCCATGAAGCGTCAGTTAGGCTAGGACCCAGCTTTCTTGTGTA
 0 ++++++
 3' TGCTCCGAATACTATAACGGAACGCGTTACGGTACTTCGCAGTCAATCCGATCCTGGGTCGAAAGAACAT

7420

BGT03_T01c

attB2

BGT03_T01c-pDONR221

Y E A Y D I A L R N A M K R Q L G
 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732

5' CAAAGTGGTTGATatcggatccgaattcgagctccgctcgacaagcttgccggccgcactcgcagcaccacca
 0 ++++++
 3' GTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacgcggcgtgagctcgtgggtggt

7490

BamHI

EcoICRI
SacI

HindIII

NotI
EagI

PspXI
XhoI
AvaI
BmeT110I

attB2

5' ccaccaccactgagatccggctgctaacaagcccgaaggaagctgagttggctgctgccaccgctgag
 0 ++++++
 3' ggtggtggtgactctaggccgacgattgttccgggctttccttcgactcaaccgacgacggtggcgactc

7560

BlnI

T7 T...tor

5' caataactagcataacccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaacta
 0 ++++++
 3' gttattgatcgattgggggaaccccggagatttgcccagaactccccaaaaaacgactttcctccttgat

7630

T7 Terminator

5' tatccggat
 0 ++++++
 3' ataggccta

7639