

o

5' **PshAI**
gcgacgatagtcattgccccgcgccaccggaaggagctgactgggttgaaggctctcaagggcatcggtc
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' cgctgctatcagtacggggcgcggtggccttctctcgactgacccaacttccgagagttcccgtagccag 3710

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5' gagatccccggtgcctaataatgagtgagctaacttacattaattgcggttgcgctcactgcccgccttccagtc
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' ctctagggccacggattactcactcgattgaatgtaattaacgcaacgcgagtgacggggcgaaagggtcag 3780

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5' **BsaXI'**
gggaaacctgctcgtgccagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattggg
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' cccttgggacagcacggctcgacgtaattacttagccggttgcgcgccctctccgccaacgcataacc 3850

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5' **BsaXI**
cgccagggtgggttttctttcaccagtgagacgggcaacagctgattgccttcaccgcctggccctga
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' gcggtcccacaaaaagaaaagtggtcactctgcccggtgtcgactaacgggaagtggcggaccgggact 3920

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5' **HpaI**
gagagttgcagcaagcgggtccacgctgggttgccccagcaggcgaaaatcctggttgatggtggttaacg
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' ctctcaacgctgcttcgccagggtgcgaccaaacggggtcgtccgcttttaggacaaactaccaccaattgc 3990

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5' gcgggatataacatgagctgtcttcggtatcgtcgtatcccactaccgagatatccgcaccaacgcgcag
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' cgccctatatgtactcgacagaagccatagcagcatagggtgatggctctataggcgtggttgcgcgctc 4060

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5' **BssHII**
cccggactcggtaatggcgcgcattgcgcccagcgcacatctgatcgttggcaaccagcatcgcagtggga
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' gggcctgagccattaccgcgcgtaacgcgggtcgcggttagactagcaaccggttggtcgtagcgtcaccct 4130

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5' acgatgccctcattcagcatttgcatgggttgttgaaaaccggacatggcactccagtcgccttcccgtt
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' tgctacgggagtaagtcgtaaacgtaccaaacactttggcctgtaccgtgaggtcagcgggaagggcaa 4200

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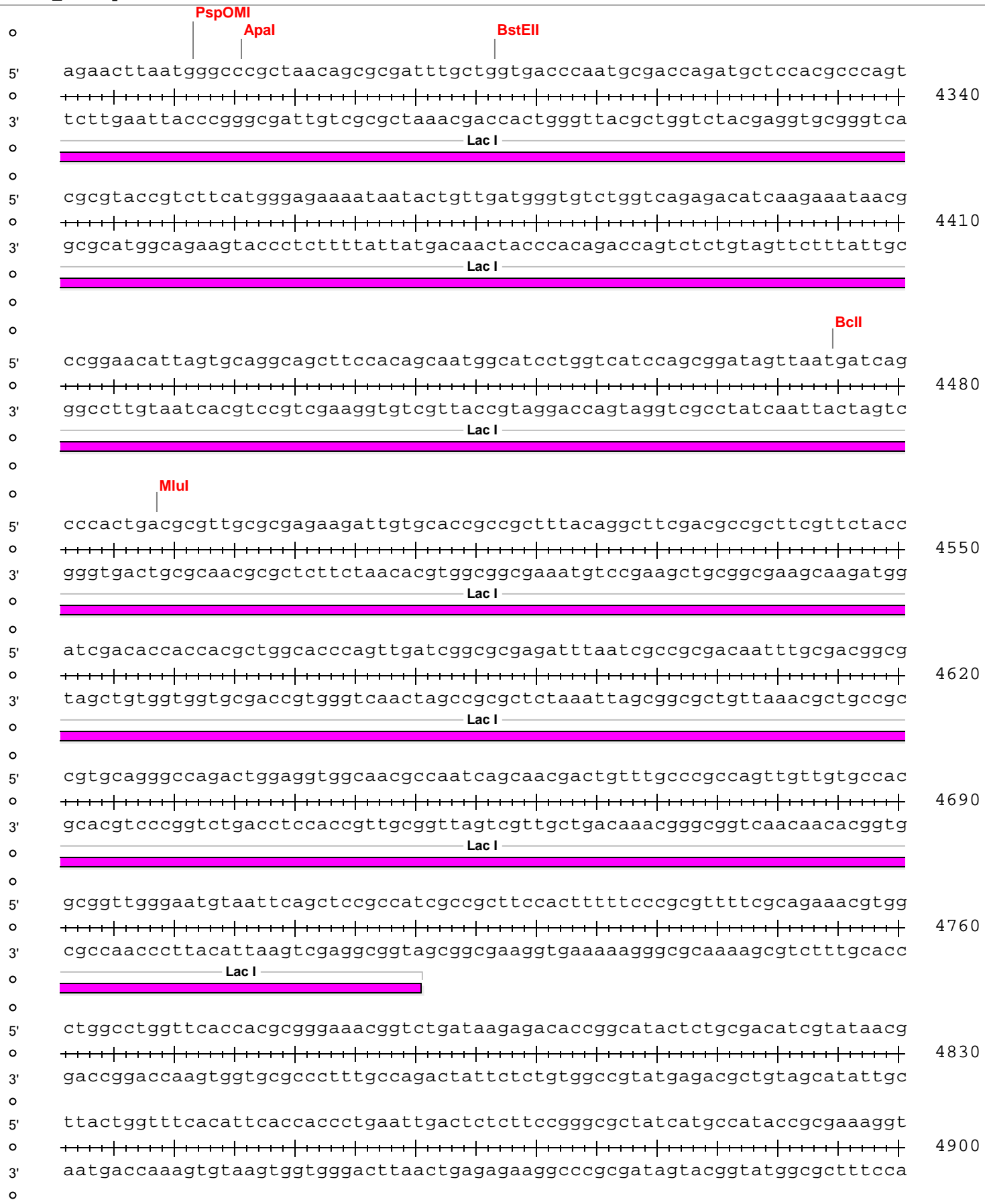
5' ccgctatcggctgaatttgattgcgagtgagatatttatgccagccagccagacgcagacgcgcggagac
+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
3' ggcgatagccgactaaactaacgctcactctataaatacgggtcggtcggctctgcgtctgcgcggctctg 4270

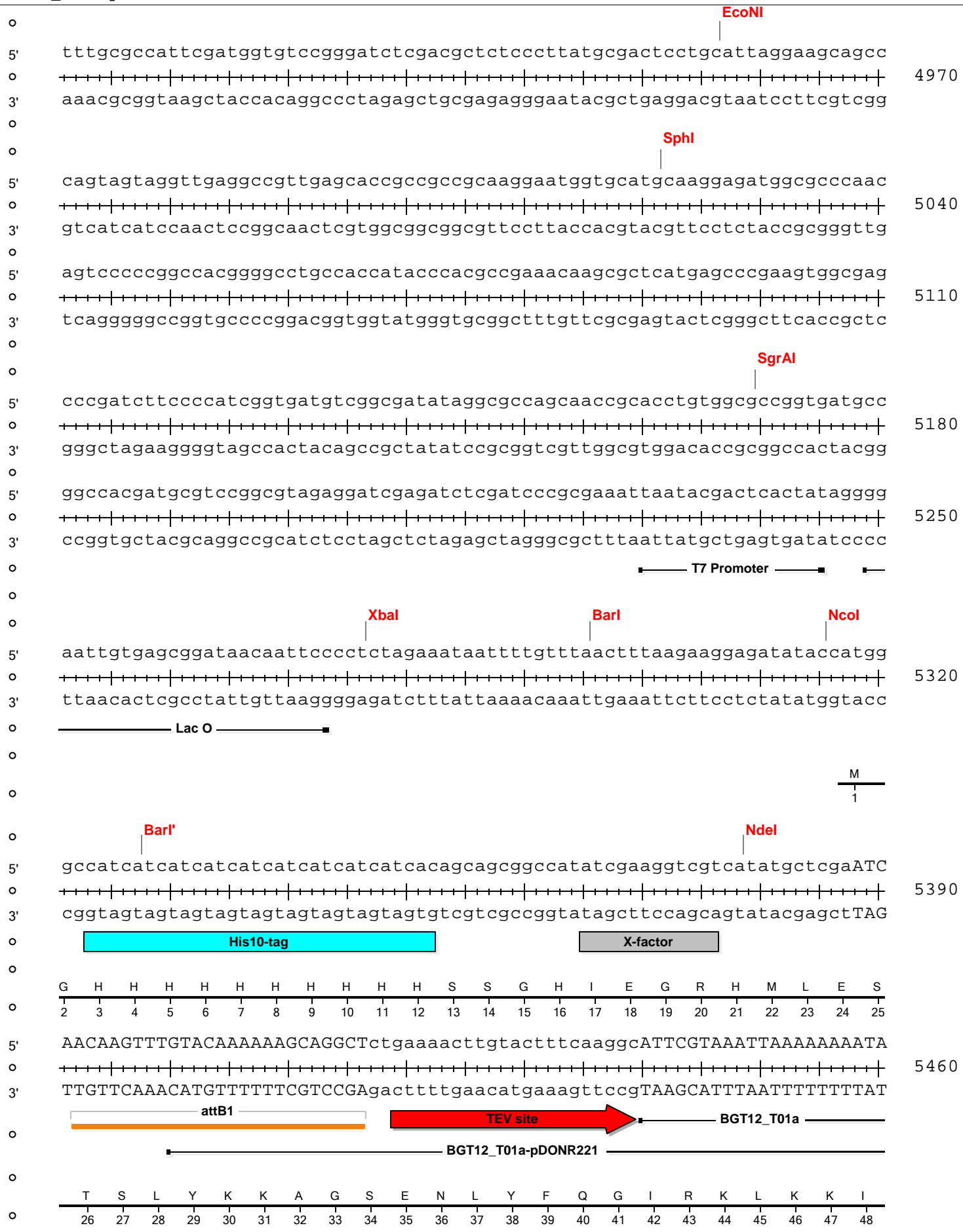
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BGT12_T01a-pET16-DEST

5' CTATTTAGATATTTAAAAACCGGAAAGCTGGTTTTTATCGGAAAGCCTGTATACAAAGCGATTATAACTA
 3' GATAAATCTATAAATTTTTGGCCTTTCGACCAAAAATAGCCTTTCGGACATATGTTTCGCTAATATTGAT

5530

BGT12_T01a

BGT12_T01a-pDONR221

L F R Y L K T G K L V F I G K P V Y K A I I T
 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71

Agel

5' TTGAACTTTTATTTTCATCGTTTGGTTGATTTGTGTGCTCGAACCAGTTCTGGTTCACAGGATAATCTTAA
 3' AACTTGAAAATAAAGTAGCAAACCAACTAAACACACGAGCTTGGCCAAGACCAAGTGTCTTATTAGAATT

5600

BGT12_T01a

BGT12_T01a-pDONR221

I E L L F H R L V D L C A R T G S G S Q D N L N
 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' TTCTGATTTAACTGTCCTTGTCAAGACCTTTGAAAGACCTCGAACGGTAAAACGGCTTATTTAAAAGCATA
 3' AAGACTAAATTGACAGGAACAGTTCTGGAACTTTCTGGAGCTTGCCATTTTGCCGAATAATTTTCGTAT

5670

BGT12_T01a

BGT12_T01a-pDONR221

S D L T V L V K T F E R P R T V K R L I K S I
 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118

Bsu36I

5' AAAAGGTTTTATCCCAACCTTAGGATTATCTTGTGACGATAGTAAATATCCTGTTCAAAATCAATGGAA
 3' TTTTCCAAAATAGGTTTGAATCCTAATAAGAACAACCTGCTATCATTTATAGGACAAGTTTAGTTACCTT

5740

BGT12_T01a

BGT12_T01a-pDONR221

K R F Y P N L R I I L V D D S K Y P V Q I N G
 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

5' TAGAACTGTGGTTATGCCATTTGACAGCGGTATATCCGCCGGAAGGAATAAAGCGTTATCACTGGTTAA
 3' ATCTTTGACACCAATACGGTAACTGTGCGCCATATAGCGGCCTTCCTTATTTGCAATAGTGACCAATT

5810

BGT12_T01a

BGT12_T01a-pDONR221

I E T V V M P F D S G I S A G R N K A L S L V K
 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165

BGT12_T01a-pET16-DEST

5' AACAGAATTTGTTTTAGTGCTGGACGATGATTTTGTTTTTTTTGTAGAAAAACCGACCTTGTC AATGCCCTT
 ++++++
 3' TTGTCTTAAACAAAATCACGACCTGCTACTAAAACAAAAAAATCTTTTTGGCTGGAACAGTTACGGGAA

5880

BGT12_T01a

BGT12_T01a-pDONR221

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

5' AAAAGAATTAAGCCAATCCAAATATTGATATTTTAGGGGCGAAGTCATAGATCTTCCATTTTACAAAA
 ++++++
 3' TTTTCTTAATTTTCGGTTAGGTTTATAACTATAAAATCCCCCGCTTCAGTATCTAGAAGGTAAAATGTTTT

5950

BGT12_T01a

BGT12_T01a-pDONR221

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

5' CCTTTGACTACTATACAGCTCCCATATTTGCAACAGTGGCTCAACCGACAATGCCAACTGGATCATTAAT
 ++++++
 3' GGAAACTGATGATATGTTCGAGGGTATAAACGTTGTCACCGAGTTGGCTGTTACGGTTGACCTAGTAATTA

6020

BGT12_T01a

BGT12_T01a-pDONR221

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

5' TGATGGTATGCCTGTGCTTGACAAGGTTGCAAATTTTTATATCGGCAGGACCCATGCTATAAAAAAATC
 ++++++
 3' ACTACCATACGGACACGAACTGTTCCAACGTTTAAAAATATAGCCGTCCTGGGTACGATATTTTTTTTGTAG

6090

BGT12_T01a

BGT12_T01a-pDONR221

D G M P V L D K V A N F Y I G R T H A I K K I
 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258

5' AAATGGGATAACCTATTA AAAAGGTTAGATCATGCTGACTTTTTTACCAGGGCCAAAGGCGTTTTGACAT
 ++++++
 3' TTTACCCTATTGGATAATTTTTCCAATCTAGTACGACTGAAAAAATGGTCCCGGTTTCCGCAAAACTGTA

6160

BGT12_T01a

BGT12_T01a-pDONR221

K W D N L L K R L D H A D F F T R A K G V L T
 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281

BGT12_T01a-pET16-DEST

5' CTGTTTAAACAGATGAGTTTAAATGTCTTCATGCCAGAACCATTTATAATAGAACTTATATGAGTTTCCG
 6230
 3' GACAAAATTGTCTACTCAAATTTACAGAAGTACGGTCTTGGTAAATATTATCTTGAATATACTCAAAGGC

BGT12_T01a

BGT12_T01a-pDONR221

S V L T D E F K C L H A R T I Y N R T Y M S F R
 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' AAACAATCTAAAAAGAGATCAGATATACTTGTTCCTAAAATACAACCATGAATCTAATATTTAAAACCCCT
 6300
 3' TTTGTTAGATTTTCTCTAGTCTATATGAACAAAGATTTTATGTTGGTACTTAGATTATAATTTTGGGA

BGT12_T01a

BGT12_T01a-pDONR221

N N L K R D Q I Y L F L K Y N H E S N I K N P
 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328

BamHI

5' CAGATGCCATAGGACCCAGCTTCTTGTACAAAGTGGTTGATtcgaggatccggctgctaacaagcccg
 6370
 3' GTCTACGGTATCCTGGGTCGAAAGAACATGTTTCACCAACTAagctcctaggccgacgattgtttcgggc

BGT1...01a

attB2

BGT12_T01a-pDONR221

Q M P
 329 330 331 332

BlnI

5' aaaggaagctgagttggctgctgccaccgctgagcaataactagcataacccttggggcctctaaacgg
 6440
 3' tttccttcgactcaaccgacgacggtggcgactcgttattgatcgtattggggaaccccgagatttgcc

T7 Terminator

5' gtcttgaggggttttttgctgaaaggaggaactatatccggatatcccgcaagaggcccggcagtaccgg
 6510
 3' cagaactccccaaaaaacgactttcctccttgatataggcctataggcggttctccgggcccgtcatggcc

T7 Terminator

5' cataaccaagcctatgcctacagcatccagggtgacggtgcccaggatgacgatgagcgcattgtagat
 6580
 3' gtattggttcggatacggatgtcgtaggtcccactgccacggctcctactgctactcgcgtaacaatcta

HindIII

ClaI

5' ttcatacacggtgctgactgctgtagcaatttaactgtgataaactaccgcattaaagcttatcgatga
 6650
 3' aagtatgtgccacggactgacgcaatcgtaaattgacactatttgatggcgtaatttcgaatagctact

o
5' taagctgtcaaaca**EcoRI**tgagaa
o +++++|++++|++++|++++|
3' attcgacagtttgtactctt
o

6670