

BGH35_B04a-pET32-DEST

5' tggcgaatgggacgcgccctgtagcggcgccattaagcgcggcggtgtggtggttacgcgcagcgtgacc 70
 0 ++++++
 3' accgcttaccctgcgcggggacatcgccgcgtaattcgcgccgccacaccaccaatgcgcgtcgcaactgg
 0
 5' gctacacttgccagcgccttagcgcgccgctcctttcgctttcttccttctccttctcgcacgttcgccc 140
 0 ++++++
 3' cgatgtgaacggctcgcgggatcgcgggcgaggaaagcgaagaagggaaggaaagagcgggtgcaagcggc
 0
 5' gctttccccgtcaagctctaaatcgggggctccctttagggttccgatttagtgctttacggcacctcga 210
 0 ++++++
 3' cgaaaggggagcttcgagatttagcccccgagggaatcccaaggctaaatcacgaaatgcccgtggagct

Dralll

|

5' ccccaaaaaacttgattaggggtgatgggtcacgtagtgggcatcgccctgatagacgggtttttcgccct 280
 0 ++++++
 3' ggggttttttgaactaatccactaccaagtgcataccccggtagcgggactatctgccaaaagcggga

AloI'

AloI

|

|

5' ttgacggtggagtccacggttctttaatagtggactcttgttccaaactggaacaacactcaaccctatct 350
 0 ++++++
 3' aactgcaacctcaggtgcaagaaattatcacctgagaacaagggttgaccttgttgtgagttgggataga

Psil

|

5' cggctctattcttttgatttataagggattttgccgatttcggcctattgggttaaaaaatgagctgattta 420
 0 ++++++
 3' gccagataagaaaactaaatattccctaaaacggctaaagccggataaccaatttttactcgactaaat

5' acaaaaatttaacgcgaattttaacaaaatattaacgcttacaatttaggtggcacttttcggggaaatg 490
 0 ++++++
 3' tgtttttaaattgcgcttaaaattgttttataattgcaaatgttaaatccaccgtgaaaagcccctttac

5' tgcgcggaaccctatttgtttattttctaaatacattcaaatatgtatccgctcatgagacaataacc 560
 0 ++++++
 3' acgcgccttggggataaacaataaaaagatttatgtaagtttatacataggcgagtactctgttattgg

5' ctgataaatgcttcaataatattgaaaaaggaagagtatgagattcaacatttcggtgtcgccttatt 630
 0 ++++++
 3' gactatttacgaagttattataacttttcttctcatactcataagttgtaaaaggcacagcgggaataa

Ap



5' cccttttttgccgcatcttgccttctgtttttgctcaccagaaacgctggtgaaagtaaaagatgctg 700
 0 ++++++
 3' gggaaaaaacgccgtaaaacggaaggacaaaaacgagtgggtctttgcgaccactttcattttctacgac

Ap



5' aagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaagatccttgagagttt 770
 0 ++++++
 3' ttctagtcaaccacgtgctcacccaatgtagcttgacctagagttgtcgccattctaggaactctcaaa

Ap



BGH35_B04a-pET32-DEST

5' tcgccccgaagaacgttttccaatgatgagcactttttaagttctgctatgtggcgcggtattatcccgt
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' agcggggcttcttgcaaaaggttactactcgtgaaaatttcaagacgatacaccgcgcataatagggca

 Ap

840

5' attgacgccgggcaagagcaactcggctcgccgcatacactattctcagaatgacttgggtgagtactcac
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' taactgcccgccttctcgttgagccagcggcgtatgtgataagagtcttactgaaccaactcatgagtg

 Ap

910

5' cagtcaagaaaagcatcttacggatggcatgacagtaagagaattatgcagtgctgccataaccatgag
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
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 Ap

980

5' tgataaactgccggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcac
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' actattgtgacgccggttgaatgaagactgttgctagcctcctggcttcctcgttggcgaaaaaacgtg

 Ap

1050

5' aacatgggggatcatgtaactcgcttgatcgttgggaaccggagctgaatgaagccataccaaacgacg
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' ttgtacccttagtacattgagcggaaactagcaacccttggcctcgacttacttcggtatggtttgctgc

 Ap

1120

5' agcgtgacaccacgatgcctgcagcaatggcaacaacgttgcgcaactattaactggcgaactacttac
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' tcgcactgtggtgctacggacgtcgttaccggttgttgcaacgcggttgataattgaccgcttgatgaatg

 Ap

1190

PstI

5' tctagcttcccggcaacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcg
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' agatcgaaggggcgttgtaattatctgacctacctccgctatttcaacgctcctggtgaagacgcgagc

 Ap

1260

5' gcccttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggtatcattg
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' cgggaaggccgaccgaccgacaaataacgactatthagacctcggccactcgcacccagagcgcataagtaac

 Ap

1330

Bsal

5' cagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacggggagtcaggcaactat
 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
 3' gtcgtgaccccggctctaccattcgggagggcatagcatcaatagatgtgctgcccctcagtcggttgata

 Ap

1400

AhdI

BGH35_B04a-pET32-DEST

```

5'   cgggggagaggcggtttgcgtattgggcgccagggtggtttttcttttcaccagtgagacgggcaacagct
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   gccctctccgccaaacgcataaccgcggtcccacaaaagaaaagtgggtcactctgccggttgtcga
o   -----
o   Lac I
o   -----
5'   gattgccttcaccgcctggcctgagagagttgcagcaagcgggtccacgctggtttgccccagcaggcg
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   ctaacgggaagtggcggaccgggactctctcaacgtcgttcgccagggtgcgaccaaacggggtcgtccgc
o   -----
o   Lac I
o   -----
5'   aaaatcctgtttgatgggtggttaacggcgggatataacatgagctgtcttcggtatcgtcgtatcccact
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   ttttaggacaaactaccaccaattgccgcctatattgtactcgacagaagccatagcagcatagggtga
o   -----
o   Lac I
o   -----
5'   accgagatgtccgcaccaacgcgcagcccgactcggtaatggcgcgcattgcgccagcgccatctgat
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   tggctctacaggcgtgggttgcgcgctcgggcctgagccattaccgcgctaacgcgggtcgcggtagacta
o   -----
o   Lac I
o   -----
5'   cgttggcaaccagcatcgcagtgggaacgatgccctcattcagcatttgcattggttggtaaaacccga
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   gcaaccgttggctgtagcgtcacccttgctacgggagtaagtgcgtaaacgtaccaacaacttttggcct
o   -----
o   Lac I
o   -----
5'   catggcactccagtcgccttcccgttccgctatcggctgaatttgattgagctgagatatttatgccag
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   gtaccgtgaggtcagcgggaagggaagcgatagccgacttaaactaacgctcactctataaatacggtc
o   -----
o   Lac I
o   -----
                    PspOMI
                    |
                    |
                    |
                    Apal
5'   ccagccagacgcagacgcgcggagacagaacttaatgggcccgcctaacagcgcgatttgctggtgacca
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
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o   -----
o   Lac I
o   -----
5'   atgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgggagaaaataactggtgatgggtgt
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   tacgctggtctacgaggtgcgggtcagcgcgcatggcagaagtaccctcttttattatgacaactaccaca
o   -----
o   Lac I
o   -----
5'   ctggtcagagacatcaagaaataacgccggaacattagtgcaggcagcttccacagcaatggcatcctgg
o   ++++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|+++++|
3'   gaccagtctctgtagtcttttattgcgcccttgaatcacgtccgctcgaagggtcgttacgtaggacc
o   -----
o   Lac I
o   -----

```

o
5' | |
 BclI **MluI**
5' tcatccagcggatagttaatgatcagcccactgacgcggttgcgcgagaagattgtgcaccgcccgttttac
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' agtaggtcgccctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcggcgaaatg
 Lac I

4410

o
5' aggcttcgacgcccgttctgttctacccatcgacaccaccacgctggcaccagttgatcggcgcgagattt
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' tccgaagctgCGGCGAAGCAAGATGGTAGCTGTGGTGGTGCGACCGTGGGTCAACTAGCCGCGCTCTAAA
 Lac I

4480

o
5' aatcgccgcgacaatttgcgacggcgcgctgcagggccagactggaggtggcaacgccaatcagcaacgac
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' ttagcggcgtggttaaagcgtgccgcgcacgtcccggcttgacctccaccggttgcggttagtcggttgcgtg
 Lac I

4550

o
5' tgtttgcccgcagttgttgtgccacgcggttgggaatgtaattcagctccgccatcgccgcttccactt
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' acaaacgggCGGTCAACAACACGGTGCGCCAACCCCTACATTAAGTCGAGGCGGTAGCGGCGAAGGTGAA
 Lac I

4620

o
5' tttcccgcgttttcgcagaaacgtggctggcctggttcaccacgcgggaaacggctctgataagagacacc
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' aaagggcgaaaagcgtctttgcaccgaccggaccaagtggctgCGCCCTTGGCAGACTATTCTCTGTGG
 Lac I

4690

o
0
5' |
 BstAPI
5' ggcactactctgCGACATCGTATAACGTTACTGGTTTCACATTCACCACCCTGAATTGACTCTCTTCCGGG
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' ccgatgagacgctgtagcatattgcaatgaccAAAGTGTAGTGAGTGGTGGGACTTAACTGAGAGAAGGCC
 Lac I

4760

o
5' cgcatacATGCCATACCGCGAAAGGTTTTGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTA
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' gcgatagtagggtatggcgtttccAAAACGCGGTAAGCTACCACAGGCCCTAGAGCTGCGAGAGGGAAT
 Lac I

4830

o
0
5' |
 EcoNI
5' tgcgactcctgcattaggaagcagcccagtagtaggttgaggccgttgagcaccgcccggcgaaggaatg
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' acgctgaggacgtaatccttcgctCGGGTCAATCCAACCTCCGGCAACTCGTGGCGGGCGGCGTTCCTTAC
 Lac I

4900

o
5' gtgcatgcaaggagatggcgcCCAACAGTCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAACA
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' cacgtacgttcctctaccgCGGTTGTCAAGGGGCGGTTGCCCCGACGGTGGTATGGGTGCGGCTTGT
 Lac I

4970

o
5' agcgcTcATGAGCCCgaagtggcGAGCCCgatcttcccatcggTgatgtcggcGatataggcGccagca
0 ++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' tcgcgagtactcgggcttcaccgctcgggctagaagggtagccactacagccgctatatatccgCGGTcgt
 Lac I

5040

o
 5' accgcacctgtggcgccggtgatgccggccacgatgctccggcgtagaggatcgagatcgatctcgatc
 o
 3' tggcgtggacaccgcgccactacggccggtgctacgcaggccgcacatctcctagctctagctagagctag

5110

SgrAI

XbaI

o
 5' ccgcgaaattaatacgaactcactataggggaattgtgagcggataacaattcccctctagaataatttt
 o
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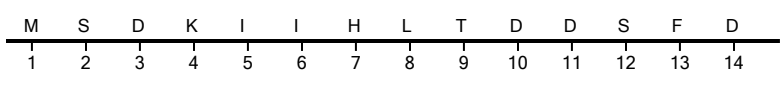
5180



o
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 o
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5250

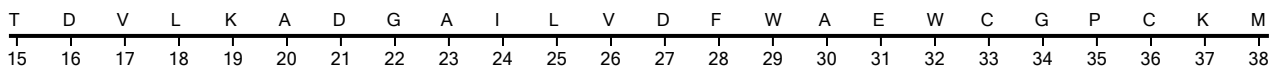
Trx Tag



o
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5320

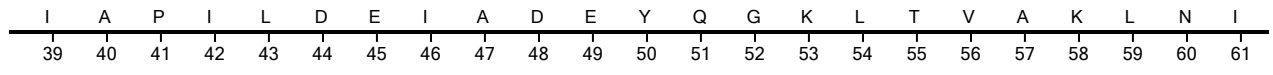
Trx Tag



o
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 o
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5390

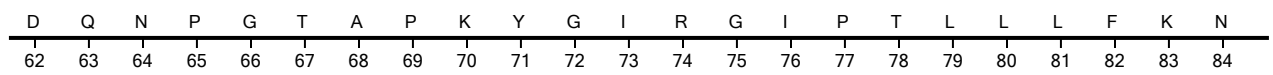
Trx Tag



o
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 o
 3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

5460

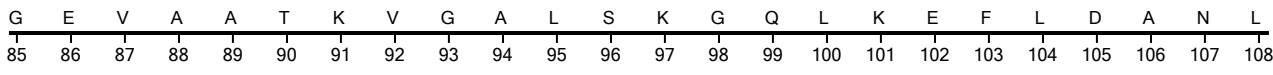
Trx Tag



o
 5' gtgaagtggcggcaaccaaagtgggtgcactgtctaaaggctcagttgaaagagttcctcgacgctaact
 o
 3' cacttcaccgcccgttggtttcaccacgctgacagatttcagtcaactttctcaaggagctgcgattgga

5530

Trx Tag



BGH35_B04a-pET32-DEST

5' ggccggttctggttctggccatatgcaccatcatcatcattcttctggtctggtgccacgcggttct
 3' ccggccaagaccaagaccggtatacgtggtagtagtagtaagaagaccagaccacggtgcgccaaga

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' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagaccatggctgc

BstBI **BglII** **Acc65I** **KpnI**

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' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

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

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

5740

5' aggcAGCCGGTTAACATGGAAGGATCAGAAATATAGGCTGGATGGCGAGGAGTTCCGGATTTTGTCCGGG
 3' tccgTCGGCCAATTGTACCTTCCTAGTCTTTATATCCGACCTACCGCTCCTCAAGGCCTAAAACAGCCCC

BseRI

BGH35_B04a **BGH35_B04a-pDONR221**

G S R L T W K D Q K Y R L D G E E F R I L S G
 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

5810

5' GCGATTCATTATTTTCGGGTTGTGCCGGAATATTGGGAGGACAGGCTGCTGAAGCTGAAGGCCTGCGGTT
 3' CGCTAAGTAATAAAAAGCCCAACACGGCCTTATAACCTCCTGTCCGACGACTTCGACTTCCGGACGCCAA

StuI

BGH35_B04a **BGH35_B04a-pDONR221**

A I H Y F R V V P E Y W E D R L L K L K A C G
 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224

5880

BGH35_B04a-pET32-DEST

5' TCAATACGGTAGAGACGTATATTCCTGTTGAAACCTGCATGAACCCCGCGAGGGAAGCTTCCGCTTCGACGG
 3' AGTTATGCCATCTCTGCATATAAGGCACCTTGGACGTAAGTGGGGCGCTCCCTTCGAAGGCGAAGCTGCC

5950

BGH35_B04a

BGH35_B04a-pDONR221

F N T V E T Y I P W N L H E P R E G S F R F D G
 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248

SfiI

PmlI

5' GTTTGC GGATGTCGCCCCGGTTCATAGAGACGGCGGGCCGGCTTGGCCTGCACGTGATTGTCCGCTCCAGC
 3' CAAACGCCTACAGCGGGCCAAGTATCTCTGCCGCCCGGCCGAACCGACGTGCACTAACAGGCAGGGTTCG

6020

BGH35_B04a

BGH35_B04a-pDONR221

F A D V A R F I E T A G R L G L H V I V R P S
 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271

5' CCTTATATATGCGCGGAGTGGGAGTTTGGCGGCTTGCCGGCCTGGCTGCTGAAATCGTCCATGGGCTTGC
 3' GGAATATATACGCGCCTCACCCCTCAAACCGCCGAACGGCCGGACCGACGACTTTAGCAGGTACCCGAACG

6090

BGH35_B04a

BGH35_B04a-pDONR221

P Y I C A E W E F G G L P A W L L K S S M G L
 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294

5' GCTGCATGGATAATGAATACCTGGAAAAAGTCGACCGCTACTATGACGAGCTTATTCGCGCCTGCTGCC
 3' CGACGTACCTATTACTTATGGACCTTTTTCAGCTGGCGATGATACTGCTCGAATAAGGCGCGGACGACGG

6160

BGH35_B04a

BGH35_B04a-pDONR221

R C M D N E Y L E K V D R Y Y D E L I P R L L P
 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318

5' GCTGCTGGATTTCGCGGGGCGGTCCGATCATTTGCGGTGCAGGTTCGAGAATGAATACGGCAGCTACGGCAAT
 3' CGACGACCTAAGCGCCCCGCCAGGCTAGTAACGCCACGTCCAGCTCTTACTTATGCCGTCGATGCCGTTA

6230

BGH35_B04a

BGH35_B04a-pDONR221

L L D S R G G P I I A V Q V E N E Y G S Y G N
 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

BGH35_B04a-pET32-DEST

5' GACACCGCGTATTTGGCTTACTTGAGGGATGGCCTGATCCGACGCGGAGTTGACTGTCTGCTGTTACCT
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' CTGTGGCGCATAAACCGAATGAACTCCCTACCGACTAGGCTGCGCCTCAACTGACAGACGACAAGTGGA

6300

————— **BGH35_B04a** —————

————— **BGH35_B04a-pDONR221** —————

D T A Y L A Y L R D G L I R R G V D C L L F T
 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364

5' CGGACGGCCCAACGACGAGATGCTGCTGGGCGGCACGGTAGAGGGGCTGCATGCAACAGTTAACTTCGG
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' GCCTGCCGGGTTGCCTGCTCTACGACGACCCGCGCGTGCATCTCCCCGACGTACGTTGTCAATTGAAGCC

6370

————— **BGH35_B04a** —————

————— **BGH35_B04a-pDONR221** —————

S D G P T D E M L L G G T V E G L H A T V N F G
 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388

5' TTCGCGCGTAGCGGAGTCCCTCGCGAAATACCGGGAGTATAGACAGGACGAACCGCTGATGGTGATGGAG
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' AAGCGCGCATCGCCTCAGGGAGCGCTTTATGGCCCTCATATCTGTCTGCTTGGCGACTACCACTACCTC

6440

————— **BGH35_B04a** —————

————— **BGH35_B04a-pDONR221** —————

S R V A E S L A K Y R E Y R Q D E P L M V M E
 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411

5' TACTGGCTTGGCTGGTTCGACCATTTGGCGGAAGCCGCATCATGTCCGGGAGGCGGGGGATGTCGCTAACG
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ATGACCGAACCGACCAAGCTGGTAACCGCCTTCGGCGTAGTACAGGCCCTCCGCCCCCTACAGCGATTGC

6510

————— **BGH35_B04a** —————

————— **BGH35_B04a-pDONR221** —————

Y W L G W F D H W R K P H H V R E A G D V A N
 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434

5' TATTGGATGAGATGCTGGAGCAAGGGGCATCCGTGAATCTCTACATGTTCCACGGCGGGACAACTTCGG
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ATAACCTACTCTACGACCTCGTTCGCCGCTAGGCACTTAGAGATGTACAAGGTGCCGCCCTGTTTGAAGCC

6580

————— **BGH35_B04a** —————

————— **BGH35_B04a-pDONR221** —————

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

BGH35_B04a-pET32-DEST

5' CTTTACAGCGGAGCCAACTACGGTGGACATTATGAACCAACGATAACAAGCTATGATTATGATGCGCCT
 6650
 3' GAAAATGTCGCCTCGGTTGATGCCACTCGTAATACTTGGTTGCTATTGTTTCGATACTAATACTACGCGGA

————— **BGH35_B04a** —————
 ————— **BGH35_B04a-pDONR221** —————

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

5' CTTACGGAGTGGGGGGATATAACCGAGAAATACAAAGCTATCCGAAGCGTGCTCGAAAAGCACGGCATA
 6720
 3' GAATGCCTCACCCCTATATTGGCTCTTTATGTTTTGATAGGCTTCGCACGAGCTTTTCGTGCCGTATG

————— **BGH35_B04a** —————
 ————— **BGH35_B04a-pDONR221** —————

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

5' CGGAAGGAGCGCCGTTCCCGGCTCCGATTCGAAGAAAGCTTACGGCAAGGTTATTTTAACGGAACGGGG
 6790
 3' GCCTTCCTCGCGGCAAGGGCCGAGGCTAAGGCTTCTTTCGAATGCCGTTCCAATAAAATTGCCTTGCCCC

————— **BGH35_B04a** —————
 ————— **BGH35_B04a-pDONR221** —————

P E G A P F P A P I P K K A Y G K V I L T E R G
 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' CGATTTGCTGGATCAGCTCGAGCAGGTATCCGCCGAACAGGTGCAATCCGTCAGCATCCGGCCGATGGAG
 6860
 3' GCTAAACGACCTAGTCGAGCTCGTCCATAGCGGCTTGTCCACGTTAGGCAGTCGTAGGCCGGCTACCTC

————— **BGH35_B04a** —————
 ————— **BGH35_B04a-pDONR221** —————

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

5' CATTACGATCAGGCTTACGGCTTTATTTTGTACTCGACCCAGGTTAAGGGGCCAAGAACGAGACAAAAGC
 6930
 3' GTAATGCTAGTCCGAATGCCGAAATAAAACATGAGCTGGGTCCAAT'TCCCCGGTTCTTGCTCTGTTTTCG

————— **BGH35_B04a** —————
 ————— **BGH35_B04a-pDONR221** —————

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

BGH35_B04a-pET32-DEST

AsiSI

5' TTCATCTTCGCGAGGTGCGCGATCGCGCTCAGGTATTCCTGGACGGCAAGCTGATCGGCGTTGTTGAGCG
 3' AAGTAGAAGCGCTCCACGCGCTAGCGCGAGTCCATAAGGACCTGCCGTTTCGACTAGCCGCAACAACCTCGC

7000

BGH35_B04a

BGH35_B04a-pDONR221

L H L R E V R D R A Q V F L D G K L I G V V E R
 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' GTGGAATCCGCAGCCGATTGAGATCGCGGTTCCCAGAGAAGGGGCAAGATTGGATGTCTCTGGTTCGAAAAT
 3' CACCTTAGGCGTCGCTAACTCTAGCGCCAAGGGTCTCTTCCCCGTTCTAACCTACAGGACCAGCTTTTA

7070

BGH35_B04a

BGH35_B04a-pDONR221

W N P Q P I E I A V P R E G A R L D V L V E N
 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621

5' ATGGGCCGCGTTAATTACGGCCCTTATCTGCGCGATCATAAAGGAATAACGGAAGGTATCCTGATCGATA
 3' TACCCGGCGCAATTAATGCCGGAATAGACGCGCTAGTATTTCCCTTATTGCCTTCCATAGGACTAGCTAT

7140

BGH35_B04a

BGH35_B04a-pDONR221

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

5' ACCAGTTCAGTCGAACTGGACGTTACCCTGCTGCCGCTTGAGTCCGAGCAGCTCGCTCGGGTCCGCTA
 3' TGGTCAAGGTCAGCTTGACCTGCCAATGGGACGACGGCGAACTCAGGCTCGTTCGAGCGAGCCCAGGCGAT

7210

BGH35_B04a

BGH35_B04a-pDONR221

N Q F Q S N W T V T L L P L E S E Q L A R V R Y
 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' TGAGTCTGTGGAAGTAACAGGGGGACAGCAGCATGACGGGCGTCTGCCTTCTATCGTGGATTTGTGGAG
 3' ACTCAGACACCTTCATTGTCCCCCTGTCTCGTACTGCCCGCAGGACGGAAGATAGCACCTAAACACCTC

7280

BGH35_B04a

BGH35_B04a-pDONR221

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

BGH35_B04a-pET32-DEST

5' GTGGATGAACCTGCCGATACGTTCCCTCCGGTTTTGACGGCTGGCAGAAGGGGATTGCCTGGATCAACGGCT
CACCTACTTGGACGGCTATGCAAGGAGGCCAAACTGCCGACCGTCTTCCCCTAACGGACCTAGTTGCCGA

7350

BGH35_B04a

BGH35_B04a-pDONR221

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

5' TCCAGCTTGGCCGCTATTGGGAAGCGGGACCGCAGCGCGCTTATACGTGCCGGTCCGCTGCTGCGCAA
AGGTGCAACCGGCGATAACCCTTCGCCCTGGCGTTCGCGCAATATGCACGGCCAGGCGACGACGCGTT

7420

BGH35_B04a

BGH35_B04a-pDONR221

F Q L G R Y W E A G P Q R A L Y V P G P L L R K
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' AGGGGAAAACGAAATTGTTTTATTTCGAGCTGCATGGCGTAACCGGACATCCCGAAGTGGATTTAACCGAT
TCCCCTTTTGCTTTAACAAAATAAGCTCGACGTACCGCATTGGCCTGTAGGGCTTCACCTAAATTGGCTA

7490

BGH35_B04a

BGH35_B04a-pDONR221

G E N E I V L F E L H G V T G H P E V D L T D
739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761

SexAI

5' GCTCCCGACCTTGGCGAGACGGCTGCGGTTGACGACCAGGTGCTAAACTTCGTACAGGAAGAATAGGACC
CGAGGGCTGGAACCGCTCTGCCGACGCCAACTGCTGGTCCACGATTTGAAGCATGTCCTTCTTATCCTGG

7560

BGH35_B04a

BGH35_B04a-pDONR221

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

BamHI

EcoRI

EcoCRI

SacI

NotI

5' CAGCTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgctcgacaagcttgcggccgcac
GTCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacgccggcgctg

7630

attB2

BG...21

5' tcgagcaccaccaccaccactgagatccggctgctaacaaagccgaaaggaagctgagttggctgc
agctcgtgggtgggtgggtggtgactctaggccgacgattgtttcgggctttccttcgactcaaccgacg

7700

o
|
BipI
5' tgccaccgctgagcaataactagcataacccttggggcctcctaaacgggtcttgaggggttttttgctg
o
++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|++++|
3' acggtggcgactcgttattgatcgtattggggaaccccggagatttgcccagaactccccaaaaaacgac

7770



o
5' aaaggaggaactatatccggat
o
++++|++++|++++|++++|++
3' tttcctccttgatataggccta
o

7792