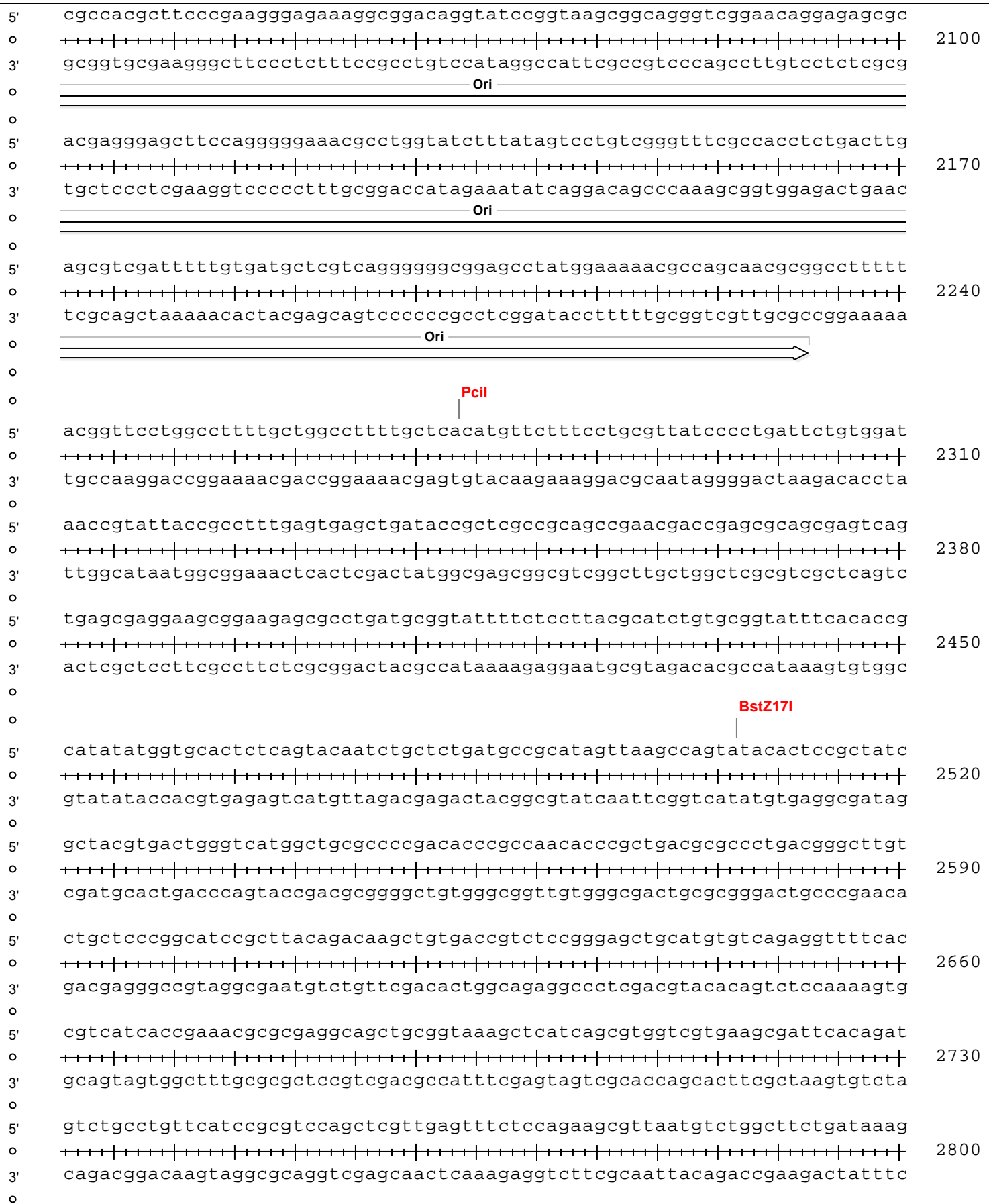
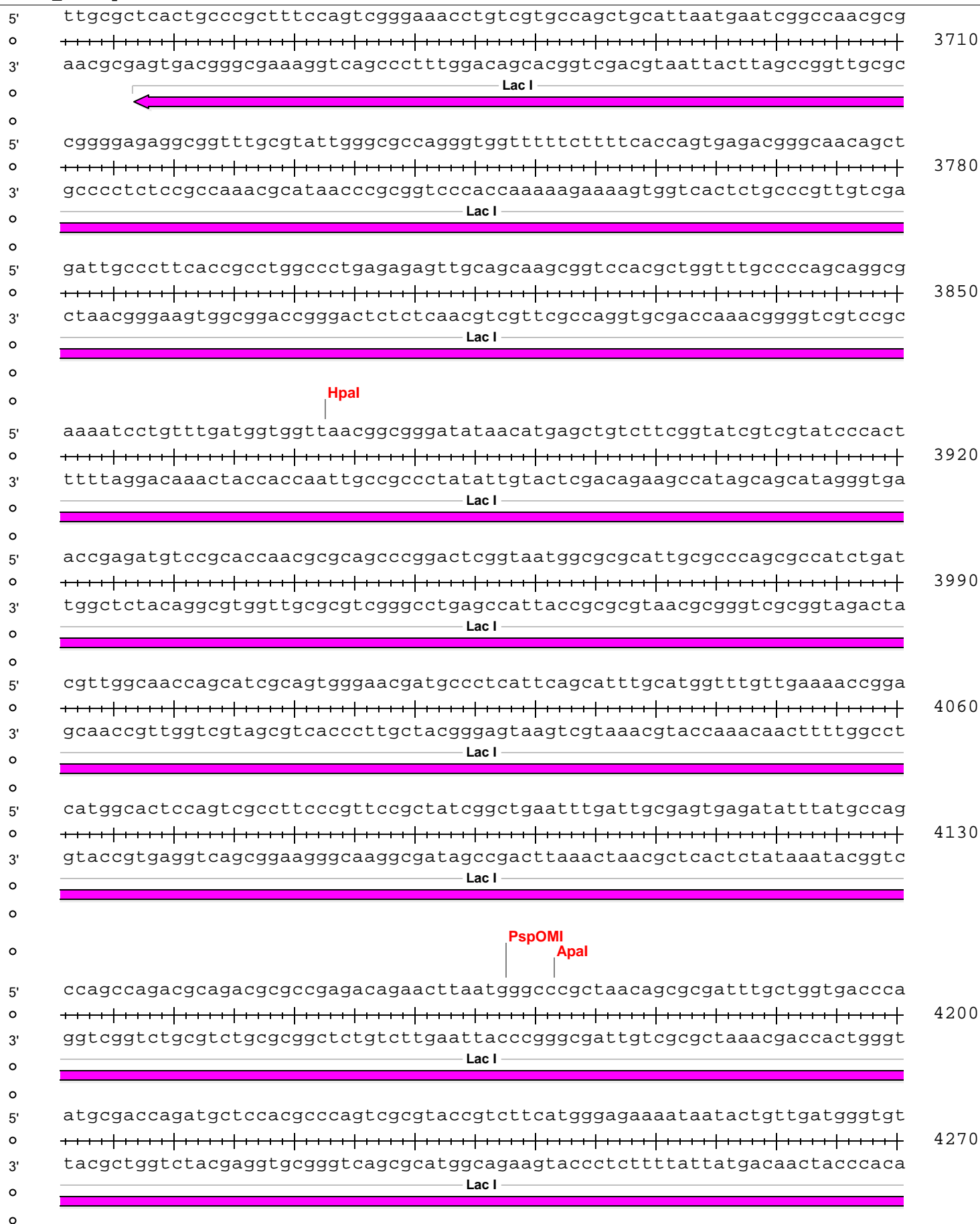


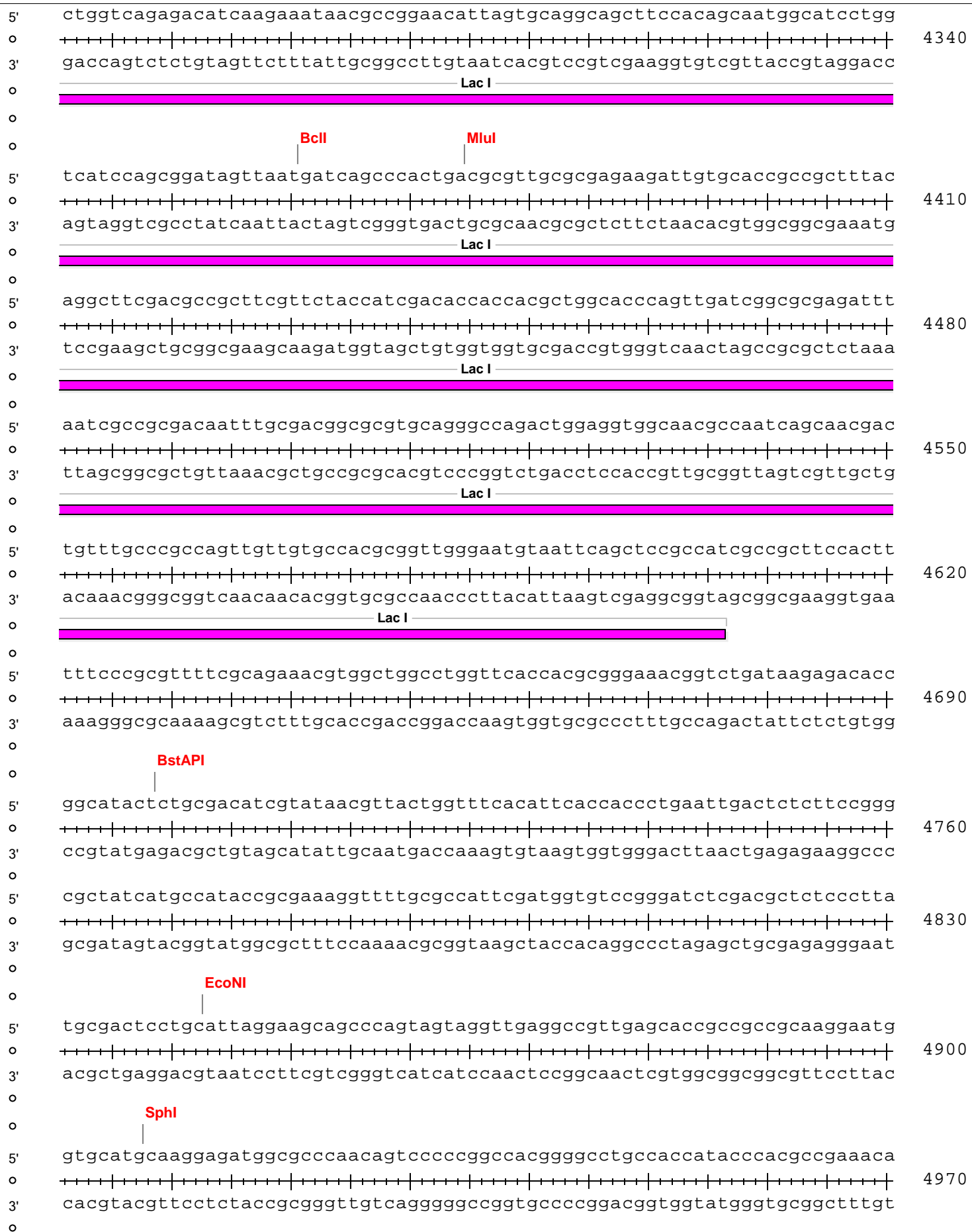
BGH33_B04c-pET32-DEST



BGH33_B04c-pET32-DEST



BGH33_B04c-pET32-DEST



BGH33_B04c-pET32-DEST

5' agcgctcatgagcccgaagtggcgagcccgatcttccccatcggtgatgtcggcgatataggcgccagca 5040
 0 ++++++
 3' tcgcgagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt

5' accgcacctgtggcgccgggtgatgccggccacgatgctcggcgtagaggatcgagatcgatctcgatc 5110
 0 ++++++
 3' tggcgtggacaccgcgccactacggccggtgctacgcaggccgcacatctcctagctctagctagagctag

XbaI

5' ccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatttt 5180
 0 ++++++
 3' ggcgctttaattatgctgagtgatataccccttaacactcgcctattgttaaggggagatctttattaaa

T7 Promoter Lac Operator

BamHI BamHI'

5' gtttaactttaagaaggagatatacatatgagcgataaaattattcacctgactgacgacagttttgaca 5250
 0 ++++++
 3' caaattgaaattcttccctctatatgtatactcgcctattttaataagtggactgactgctgtcaaaactgt

Trx Tag

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

RsrII

5' cggatgtactcaaagcggacggggcgatcctcgtcgatttctgggcagagtgggtgcgggtccgtgcaaaat 5320
 0 ++++++
 3' gcctacatgagtttcgcctgccccgctaggagcagctaaagaccgctctcaccacgccaggcacgtttta

Trx Tag

T	D	V	L	K	A	D	G	A	I	L	V	D	F	W	A	E	W	C	G	P	C	K	M
15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38

5' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaaactgaccgttgcaaaactgaacatc 5390
 0 ++++++
 3' ctagcggggctaagacctactttagcgcactgcttatagtcccgtttgactggcaacgttttgacttgtag

Trx Tag

I	A	P	I	L	D	E	I	A	D	E	Y	Q	G	K	L	T	V	A	K	L	N	I
39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61

5' gatcaaaaccctggcactgcgccgaaatatggcatccgtgggtatcccgactctgctgctgttcaaaaacg 5460
 0 ++++++
 3' ctagttttgggaccgtgacgcggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

Trx Tag

D	Q	N	P	G	T	A	P	K	Y	G	I	R	G	I	P	T	L	L	L	F	K	N
62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84

BGH33_B04c-pET32-DEST

5' gtgaagtggcggcaaccaaagtgggtgcaactgtctaaaggtcagttgaaagagttcctcgacgctaact
 3' cacttcaccgcccgttggtttcaccacgtgacagatttccagtcaactttctcaaggagctgcgattgga

5530

Trx Tag

G E V A A T K V G A L S K G Q L K E F L D A N L
 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108

5' ggccggttctgggttctggccatatgcaccatcatcatcattcttctgggtctggtgccacgcggttct
 3' ccggccaagaccaagaccggtatacgtggtagtagtagtaagaagaccagaccacgggtgcgccaaga

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
 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131

His6-tag

Thrombin

BglII

Acc65I

KpnI

5' ggtatgaaagaaccgctgctgctaaattcgaacgccagcacatggacagcccagatctgggtaccgacg
 3' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacctatggctgc

5670

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

NcoI

5' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca
 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

5740

Enterokinase

attB1

TEV site

BGH33_B04c-pDONR221

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' aggcGCCGGGGCACCCGTCCCGCCCGGCGGCGAGCCGCTCTACACGGAGCAGGACCTCGCCGTGAACGGC
 3' tccgCGGCCCCGTGGGCAGGGCGGGCCCGCTCGGCGAGATGTGCCTCGTCCTGGAGCGGCACTTGCCG

5810

T...
 BGH33_B04c
 BGH33_B04c-pDONR221

G A G A P V P P G G E P L Y T E Q D L A V N G
 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201

BspMI
BsiWI

5' AGGGAGGGCTTTCCGAAC TACCGCATCCCAGCGCTGACCGTCACGCCCCGACGGGGACCTGCTGGCCTCGT
TCCCTCCCGAAAGGCTTGATGGCGTAGGGTCGCGACTGGCAGTGCGGGCTGCCCCCTGGACGACCGGAGCA
3' 5880

BGH33_B04c

BGH33_B04c-pDONR221

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

AgeI

5' ACGACGGCCCGCCGACCGGTATCGACGCGCCCGGCCCAACTCCATCCTCCAACGCCGAGCACCGACGG
TGCTGCCGGCGGGCTGGCCATAGCTGCGCGGGCCGGGTTGAGGTAGGAGGTTGCGGCCTCGTGGCTGCC
3' 5950

BGH33_B04c

BGH33_B04c-pDONR221

Y D G R P T G I D A P G P N S I L Q R R S T 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

5' CGGCCGGACGTGGGGCGAGCAACAGGTCGTCAGCGCCGGCCAGACCACCGCGCCGATCAAGGGGTTCTCC
GCCGGCCTGCACCCCGCTCGTTGTCCAGCAGTCGCGGCCGGTCTGGTGGCGCGGCTAGTTCCCAAGAGG
3' 6020

BGH33_B04c

BGH33_B04c-pDONR221

G R T W G E Q Q V V S A G Q T T A P I K G F 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' GACCCAGCTACCTTGTTCGACCGGAAACCGGGACCATCTTCAACTTCCACGTCTACTCCCAGCGGCAGG
CTGGGGTCGATGGAACAGCTGGCCCTTTGGCCCTGGTAGAAGTTGAAGGTGCAGATGAGGGTCGCCGTCC
3' 6090

BGH33_B04c

BGH33_B04c-pDONR221

D P S Y L V D R E T G T I F N F H V Y S Q R Q
272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294

NruI

5' GCTTCGCCGGCAGCCGGCCCGGCACCGACCCGGCAGACCCCAACGTGCTCCACGCCAACGTGCGGACCTC
CGAAGCGGCCGTCGGCCGGGCGTGGCTGGGCCGTCTGGGGTTGCACGAGGTGCGGTTGCAGCGCTGGAG
3' 6160

BGH33_B04c

BGH33_B04c-pDONR221

G F A G S R P G T D P A D P N V L H A N V A T S
295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318

BGH33_B04c-pET32-DEST

SexAI

5' GACCGACGGCGGTCTGACCTGGTTCGCACCGGACCATCACGGCCGACATCACCCCGGATCCGGGCTGGCGC
 3' CTGGCTGCCGCCAGACTGGACCAGCGTGGCCTGGTAGTGCCGGCTGTAGTGGGGCCTAGGCCCGACCGCG

6230

BGH33_B04c

BGH33_B04c-pDONR221

T D G G L T W S H R T I T A D I T P D P G W R
 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

5' AGCCGCTTCGCCGCCTCCGGCGAAGGCATCCAGCTCCGCTATGGACCCACGCCGGTCTGACTCATCCAGC
 3' TCGGCGAAGCGGCGAGGCCGCTTCCGTAGGTCGAGGCGATACCTGGGGTTCGGCCAGCTGAGTAGGTCG

6300

BGH33_B04c

BGH33_B04c-pDONR221

S R F A A S G E G I Q L R Y G P H A G R L I Q
 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364

5' AGTACACGATCATCAACGCTGCCGGCGCCTTCCAGGCGGTGAGCGTGTACAGCGACGACCACGGAAGGAC
 3' TCATGTGCTAGTAGTTGCGACGGCCGCGGAAGGTCCGCCACTCGCACATGTCTGCTGCTGGTGCCTTCCCTG

6370

BGH33_B04c

BGH33_B04c-pDONR221

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

Ascl

Mrel

5' CTGGCGCGCCGGCGAAGCCGTCGGGGTCGGCATGGACGAGAACAAGACCGTGGAACTCTCCGATGGCCGG
 3' GACCGCGCGGCCGCTTCGGCAGCCCCAGCCGTACCTGCTCTTGTTCCTGGCACCTTGAGAGGCTACCGGCC

6440

BGH33_B04c

BGH33_B04c-pDONR221

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

5' GTCCTGCTCAACAGCCGCGACTCGGCCCGCAGCGGATACCGTAAGGTGGCCGCTCTCCACTGACGGCGGCC
 3' CAGGACGAGTTGTTCGGCGCTGAGCCGGGCGTTCGCTATGGCATTCCACCGGCAGAGGTGACTGCCGCCGG

6510

BGH33_B04c

BGH33_B04c-pDONR221

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

o
5' TAGGACCCAGCTTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgctcgacaagcttgcg 6930
o
3' ATCCTGGGTCGAAAGAACATGTTTCACCAACTAtagcctaggcttaagctcgaggcagctgttcgaacgc

EcoRI
EcoRI
SacI
HindIII
NotI



— BGH33_B...ONR221 —

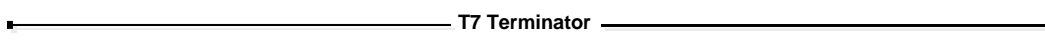
552

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PspXI
XhoI
AvaI
BmeT110I

BplI

o
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o
5' tttgctgaaaggaggaactatatccggat 7099
o
3' aaacgactttcctccttgatataggccta

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