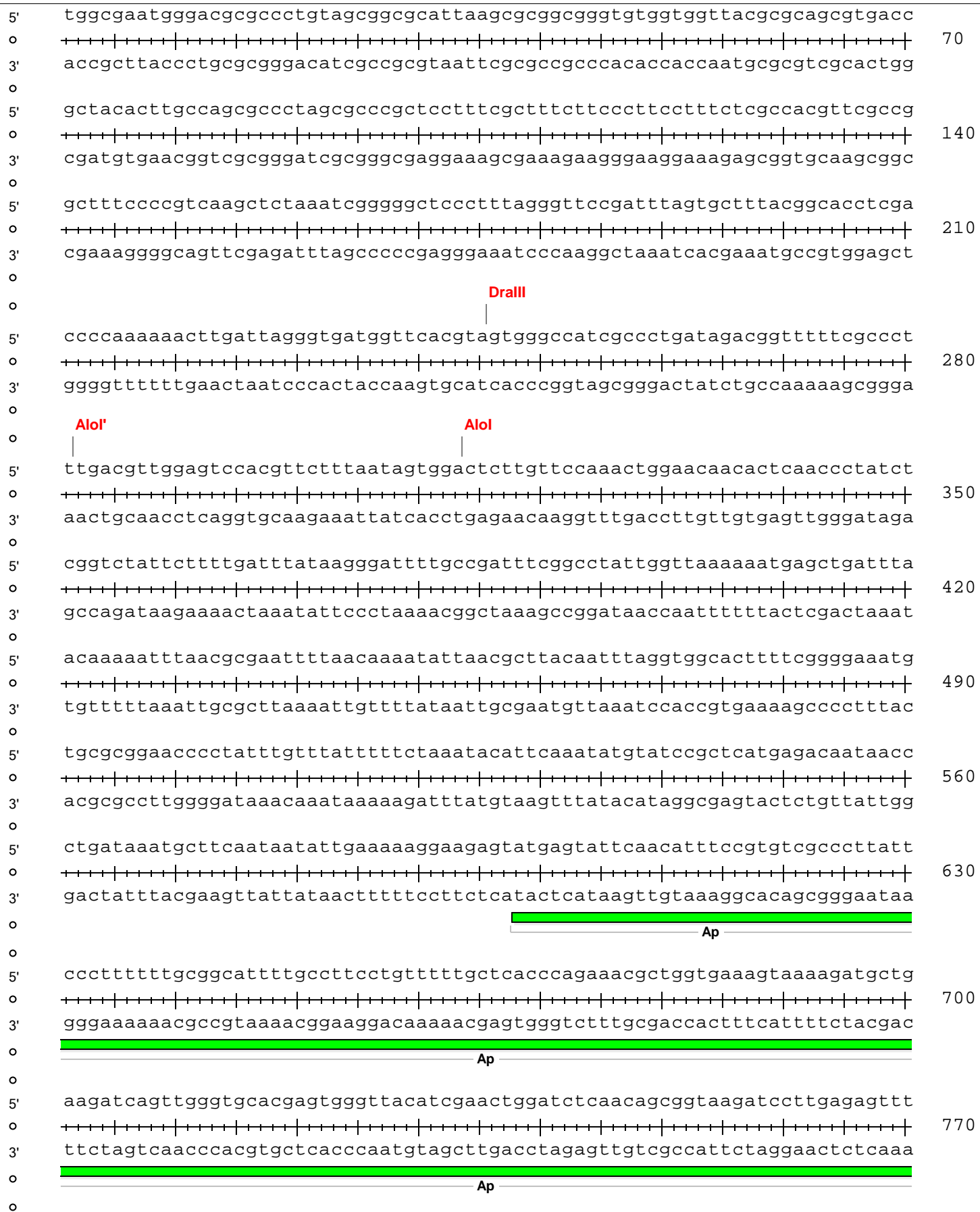
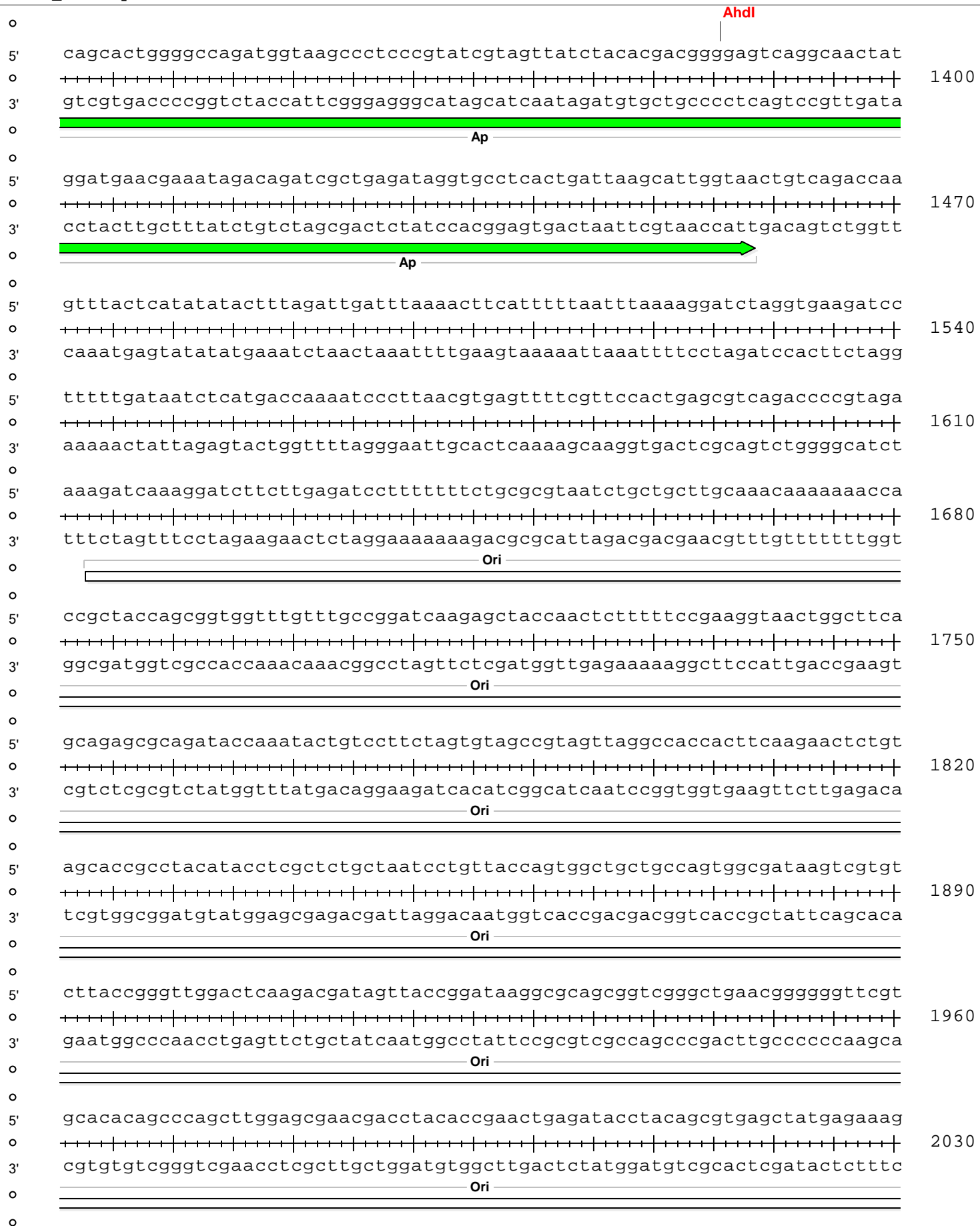


BGT10_T01b3-pET32-DEST





BGT10_T01b3-pET32-DEST

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 Ori

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 2170
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 Ori

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 Ori

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 2310
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 PciI

5' aaccgtattaccgcttttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcagtcag
 2380
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 SapI

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 BstZ17I

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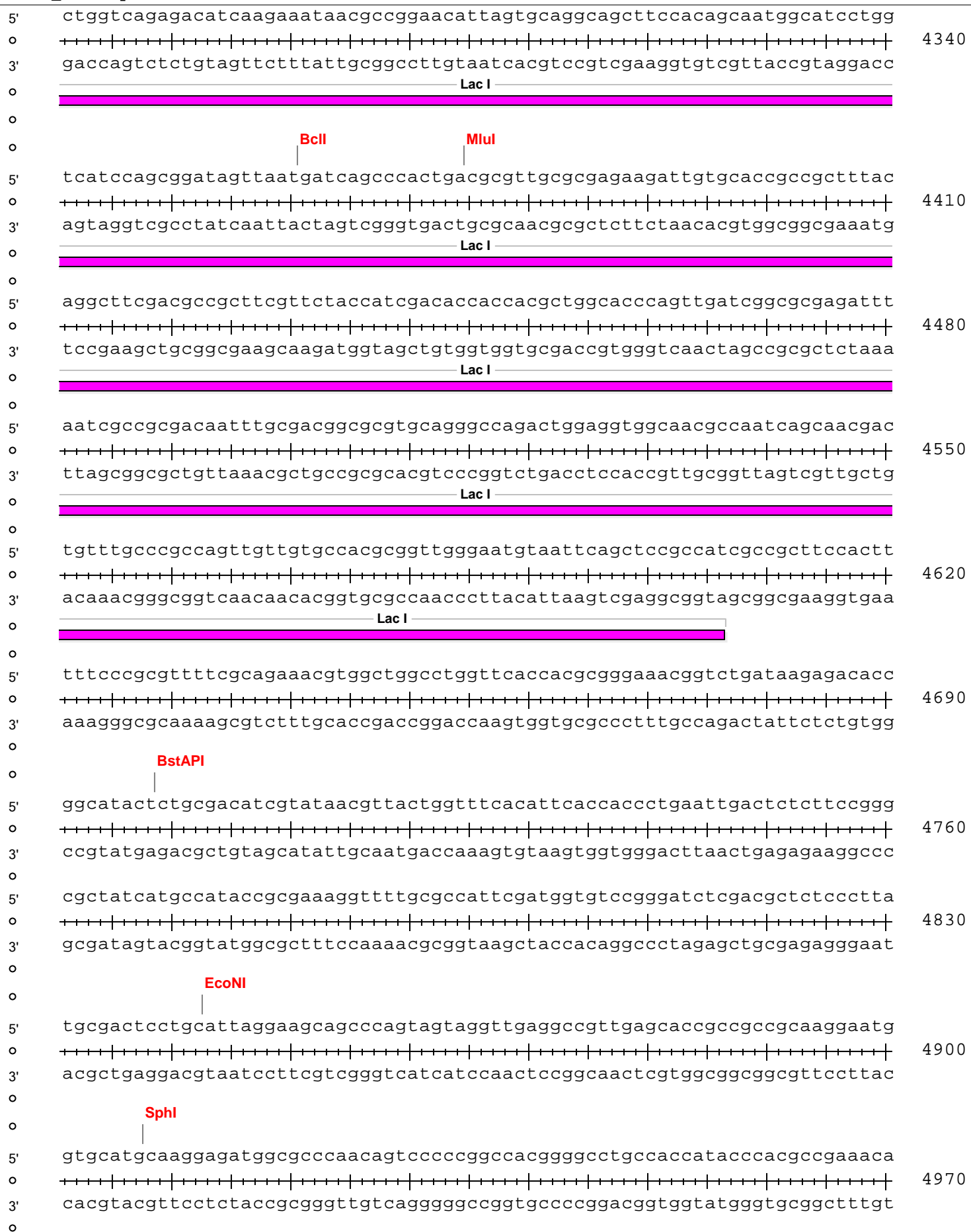
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 Tth111I

BGT10_T01b3-pET32-DEST

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o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
3'	aacgcgagtgacggggcgaagggtcagcccttggacagcacggctcgacgtaattacttagccggttg	
o		
	← Lac I	
5'	cggggagaggcggtttgcgtattggcgccagggtggttttctttcaccagtgagacgggcaacagct	3780
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
3'	gccccctctccgccaacgcataaccgcggtcccaccaaagaaagtgggtcactctgccggtg	
o		
	Lac I	
5'	gattgccttcaccgcctggcctgagagagttgcagcaagcgggtccacgctggtttgccccagcaggcg	3850
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
3'	ctaacgggaagtggcggaccgggactctctcaacgtcgcttcgccagggtgcgaccaaacggggtcg	
o		
	Lac I	
o		
	HpaI	
5'	aaaatcctgtttgatgggtggttaacggcgggatataacatgagctgtcttcggtatcgctcgatccact	3920
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
3'	tttaggacaaaactaccaccaattgcccgcctatatattgtactcgacagaagccatagcagcataggg	
o		
	Lac I	
o		
	BssHIII	
5'	accgagatgtccgcaccaacgcgcagcccggactcggtaatggcgcgcatcgcccagcgccatctgat	3990
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
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o		
	Lac I	
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o		
	Lac I	
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o		
	Lac I	
o		
	PspOMI Apal BstEII	
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o		
	Lac I	
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o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	
3'	tacgctggtctacgaggtgcgggtcagcgcattggcagaagtaccctcttttattatgacaactaccaca	
o		
	Lac I	

BGT10_T01b3-pET32-DEST



BGT10_T01b3-pET32-DEST

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 0 ++++++
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5040

5' accgcacctgtggcgccgggtgatgccggccacgatgcgctccggcgtagaggatcgagatcgatctcgatc
 0 ++++++
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5110

XbaI

5' ccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaaataatttt
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5180

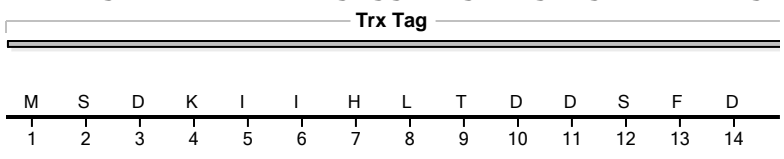


BamHI

BamHI'

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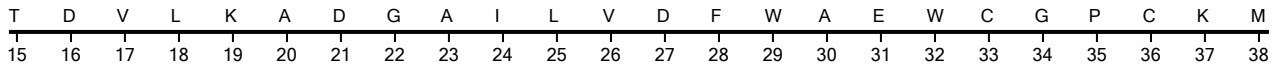
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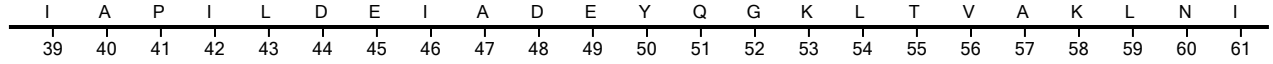
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5320



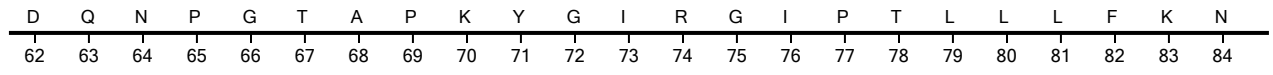
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5390



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5460



BGT10_T01b3-pET32-DEST

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 3' cacttcaccgcccgttggtttcacccacgtgacagatttccagtcaactttctcaaggagctgcgattgga
 Trx Tag

5530

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

MscI

5' ggccggttctggttctggccatatgcaccatcatcatcatcattcttctggtctggtgccacgcggttct
 3' ccggccaagaccaagaccggtatagctgtagtagtagtaagaagaccagaccacggtgcgccaaga
 T...g His6-tag Thrombin

5600

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

BglII

Acc65I

KpnI

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 3' ccatactttcttggcgacgacgatttaagcttgcggtcgtgtacctgtcgggtctagacctatggctgc
 En...e

5670

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

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 3' tgctgctgttccggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt
 Enterokinase attB1 TEV site

5740

BGT10_T01b3-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' aggcTTCCAGCCCCTATTAGACGCTTATACAGACAGCACCCGTTTAGATGAAACCGATTATAAGCCCCCA
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 Catalytic Module

5810

BGT10_T01b3
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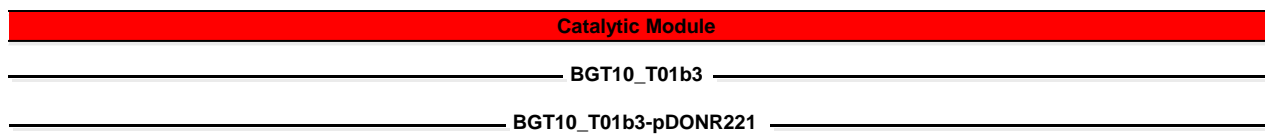
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5880

MfeI

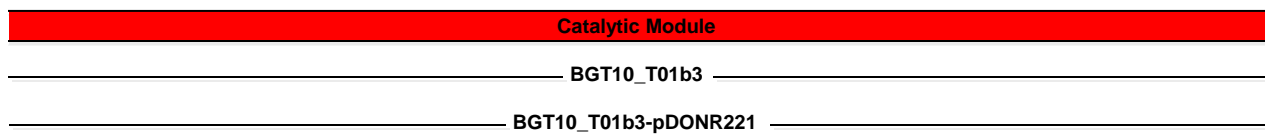


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 3' ACATAAAGTAGAATTTCGGTTGCGATGTGTTAGTGGGAGGTGGTTTTGGGATTGCTTGGGAGGCTAGAACA

5950

AfIII

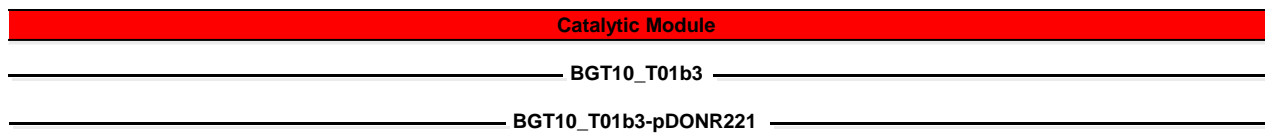


L Y F I L S Q R Y T I T L H Q N P N E P S D L V
 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248

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6020

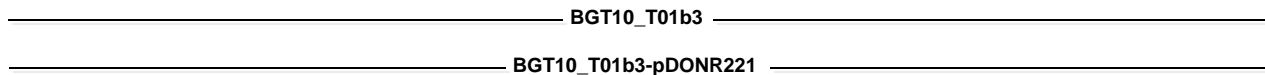
AgeI



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 3' CCACTTTTACTTCAGGGATTAAAGTTAGAGAACTAATGCGGTATCCGAACTACTTAACTGAAATCTC

6090



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

BGT10_T01b3-pET32-DEST

o

BsmI NheI BmtI
| | |

5' ATCGTTATTTGAGAATGCCTTTATATTACGCTAGCTTGCATTATAAAGCCGAGAGCGTGAATGACACCAC

o

3' TAGCAATAAACTCTTACGGAAATATAATGCGATCGAACGTAATATTTCCGGCTCTCGCACTTACTGTGGTG

6160

Catalytic Module

o

————— BGT10_T01b3 —————

————— BGT10_T01b3-pDONR221 —————

o

D R Y L R M P L Y Y A S L H Y K A E S V N D T T

o

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' CGCGCCCTACAAACTCAAAGACAACAGCCTTTATGCTTTAAAAAGCCCTCCCATCATTTTAAAGAAAAC

o

3' GCGCGGGATGTTTGAGTTTCTGTTGTCGGAAATACGAAATTTTTTCGGGAGGGTAGTAAAAATTTCTTTTG

6230

Catalytic Module

o

————— BGT10_T01b3 —————

————— BGT10_T01b3-pDONR221 —————

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A P Y K L K D N S L Y A L K K P S H H F K E N

o

319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341

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o

3' GTGGGATTAATAACGCGTCATCACTTACTACTCTCGCTAGGAACTTTTCTCCCAAACGCTCGAAACAGC

6300

Catalytic Module

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————— BGT10_T01b3-pDONR221 —————

o

H P N L C A V V N D E S D P L K R G F A S F V

o

342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364

5' CGAGCAACCCTAACGCTCCTATAAGGAACGCTTTCTATGACGCTTTAAATTCTATTGAGCCAGTTACTGG

o

3' GCTCGTTGGGATTGCGAGGATATTCCTTGCGAAAGATACTGCGAAATTTAAGATAACTCGGTCAATGACC

6370

Catalytic Module

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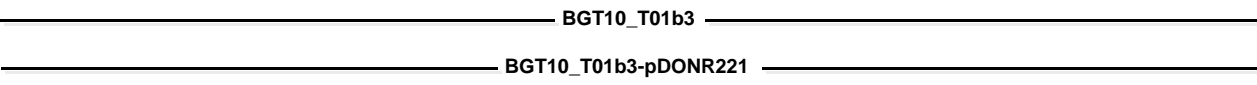
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365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388

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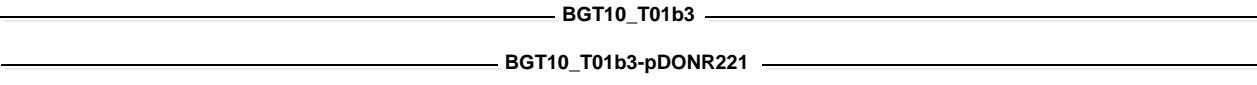
6440



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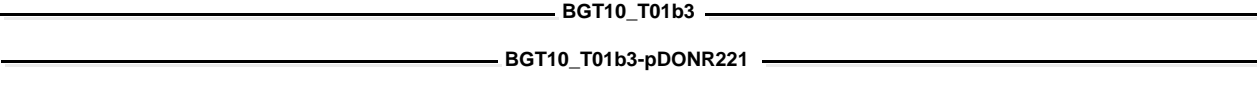
6510



F N L C F E N T Q G Y G Y V T E K I I D A Y F
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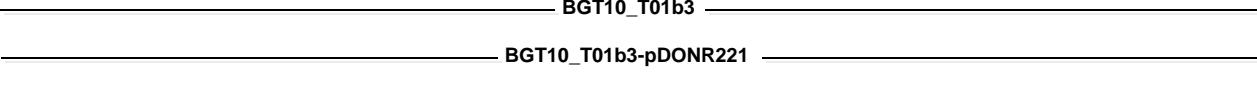
6580



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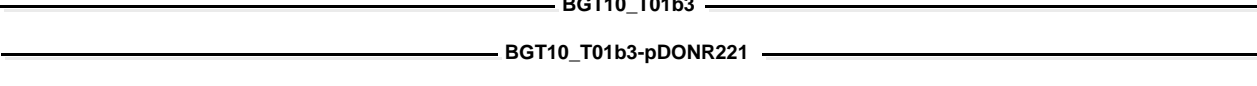
6650



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 3' ATAAATCTGTACGAGATACTTTTGGGAAATTTGTGGGAACTACCCTTTCGAATGAAAATGGTTTTAAACT

6720



Y L D M L Y E N P L N T L D G K A Y F Y Q N L
 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504

BGT10_T01b3-pET32-DEST

5' GTTTTAAAAAATCCTAGATTTTTTTTAAAACGATTTTAGAAAACGACACGATCTATCACGATAACCCTTT
 0 ++++++
 3' CAAAATTTTTTTAGGATCTAAAAAATTTTGCTAAAAATCTTTTGCTGTGCTAGATAGTGCTATTGGGAAA

Catalytic Module

0 _____ **BGT10_T01b3** _____

0 _____ **BGT10_T01b3-pDONR221** _____

0

0 S F K K I L D F F K T I L E N D T I Y H D N P F

0 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528

6790

5' CATTTCCTATCGCGATTTGAATGAGCCTTTAGTAGCTATTGATGATTTGAGGGTTAATTATGATGATTG
 0 ++++++
 3' GTAAAAGATAGCGCTAAACTTACTCGGAAATCATCGATAACTACTAAACTCCCAATTAATACTACTAAAC

Catalytic Module

0 _____ **BGT10_T01b3** _____

0 **DDLRVNY Repeats**

0 _____ **BGT10_T01b3-pDONR221** _____

0

0 I F Y R D L N E P L V A I D D L R V N Y D D L

0 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551

6860

5' AGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTGAGGGTTA
 0 ++++++
 3' TCCCAATTAATACTACTAAACTCCCAATTAATACTACTAAACTCCCAATTAATACTACTAAACTCCCAAT

BGT10_T01b3

DDLRVNY Repeats

0 _____ **BGT10_T01b3-pDONR221** _____

0

0 R V N Y D D L R V N Y D D L R V N Y D D L R V

0 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574

6930

5' ATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATTAGGACCCAGCTTTCTTGACAA
 0 ++++++
 3' TAATACTACTAAACTCCCAATTAATACTACTAAACTCCCAATTAATAATCCTGGGTCGAAAGAACATGTT

BGT10_T01b3

attB2

DDLRVNY Repeats

0 _____ **BGT10_T01b3-pDONR221** _____

0

0 N Y D D L R V N Y D D L R V N Y

0 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591

7000

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 0 ++++++
 3' TCACCAACTatagcctaggcttaagctcgaggcagctgttcgaacgccggcggtgagctcgtggtggtggt

attB2

BamHI **EcoRI** **EcoCRI** **SacI** **Sall** **NotI** **EagI** **PspXI** **XhoI** **AvaI** **BmeT110I**

7070

BGT10_T01b3-pET32-DEST

Bipl

o

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o

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← T7 Terminator →

o

5' taactagcataacccttggggcctctaaacgggtcttgaggggttttttgctgaaaggaggaactatat 7210

o

3' attgatcgtattggggaaccccggagattgcccagaactccccaaaaaacgactttcctccttgatata

o

← T7 Terminator →

o

5' ccggat

o

3' ggcta

o

7216