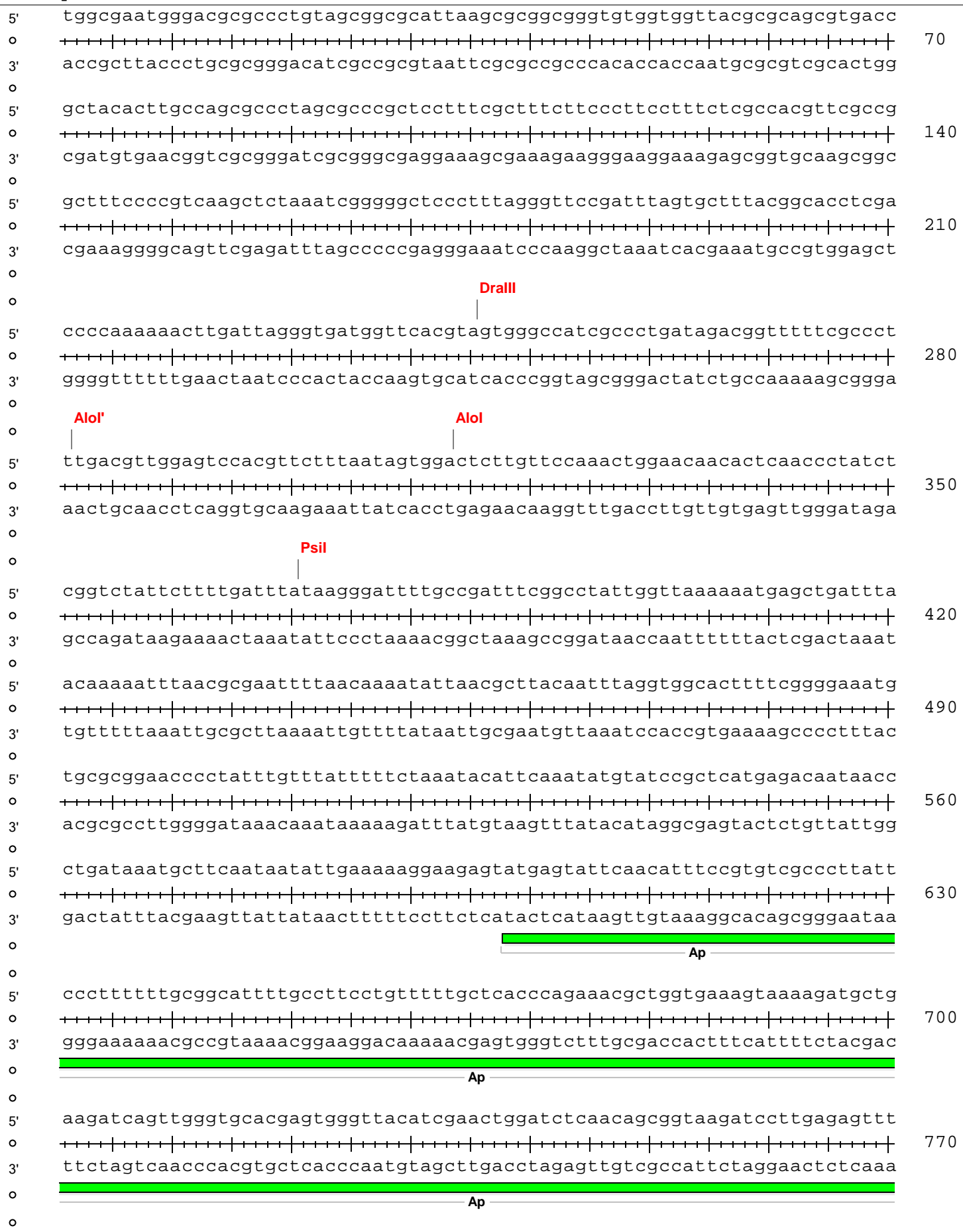
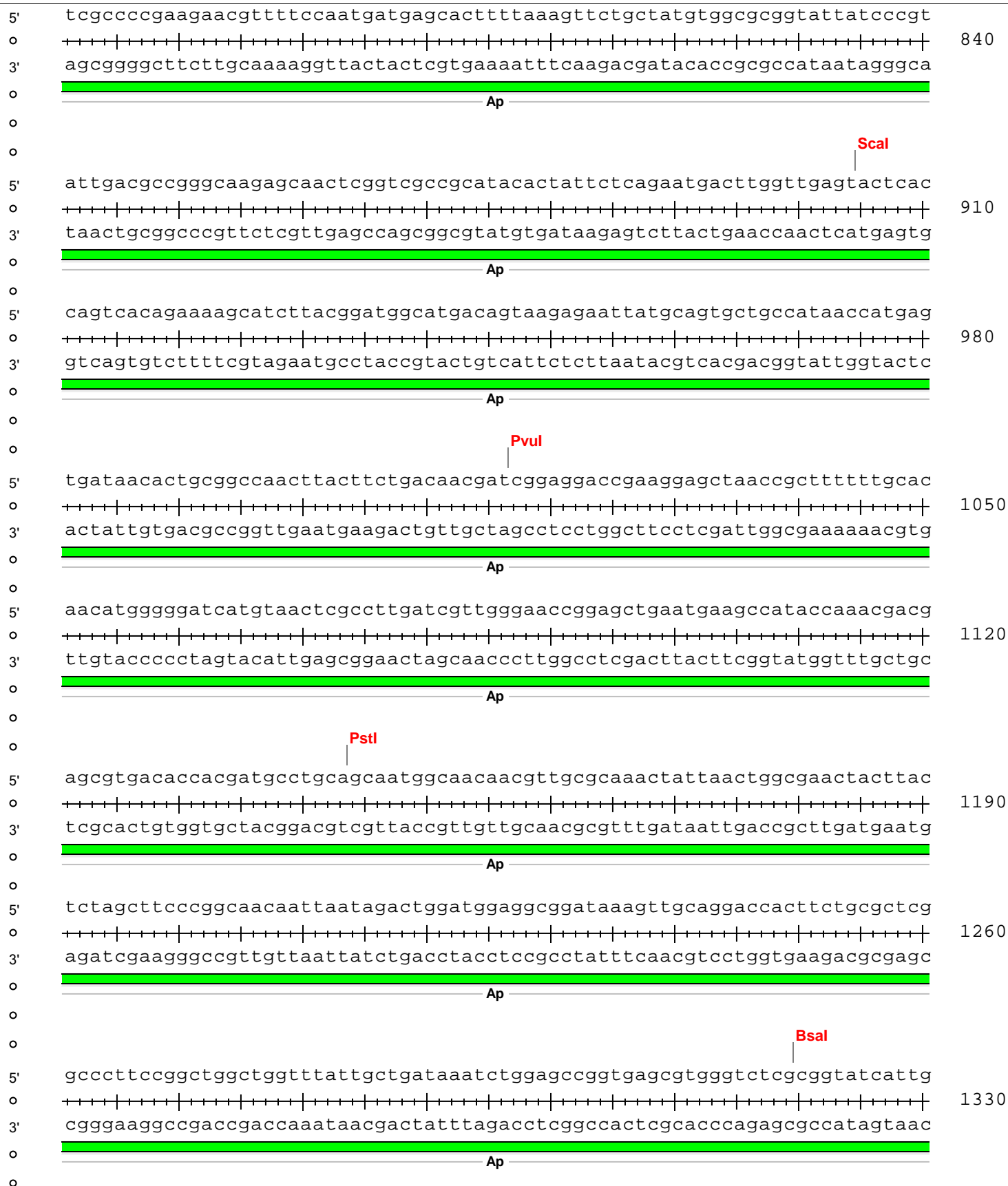


B01a2-pET32-DEST



B01a2-pET32-DEST



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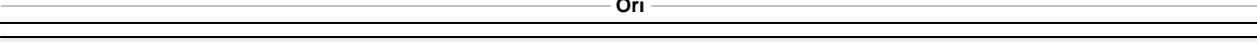
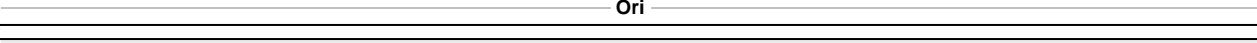
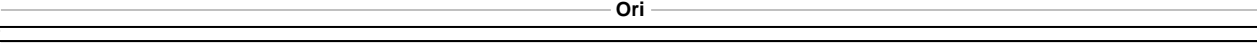
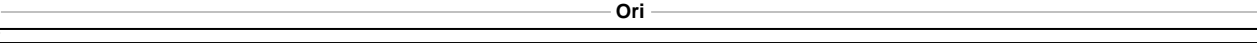
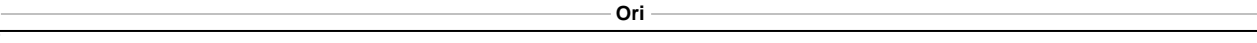
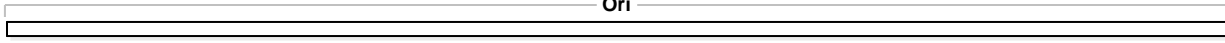
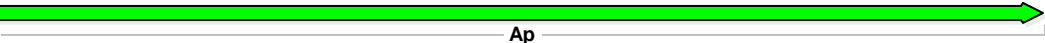
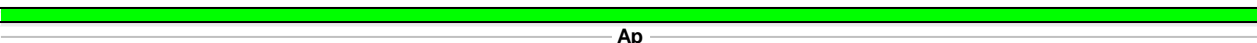
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Ori

o

AhdI



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 Ori

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 Ori

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 Ori

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 PciI

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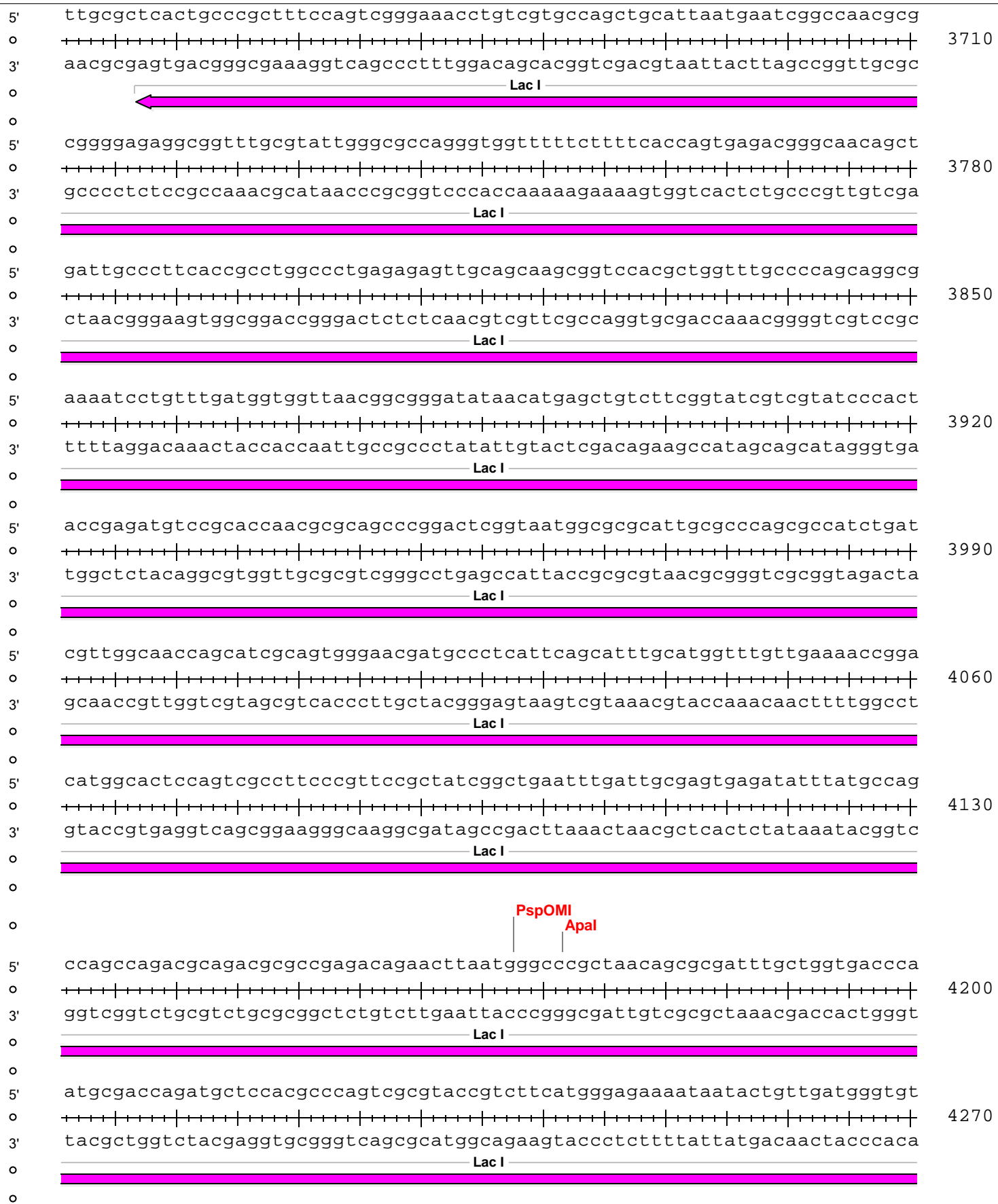
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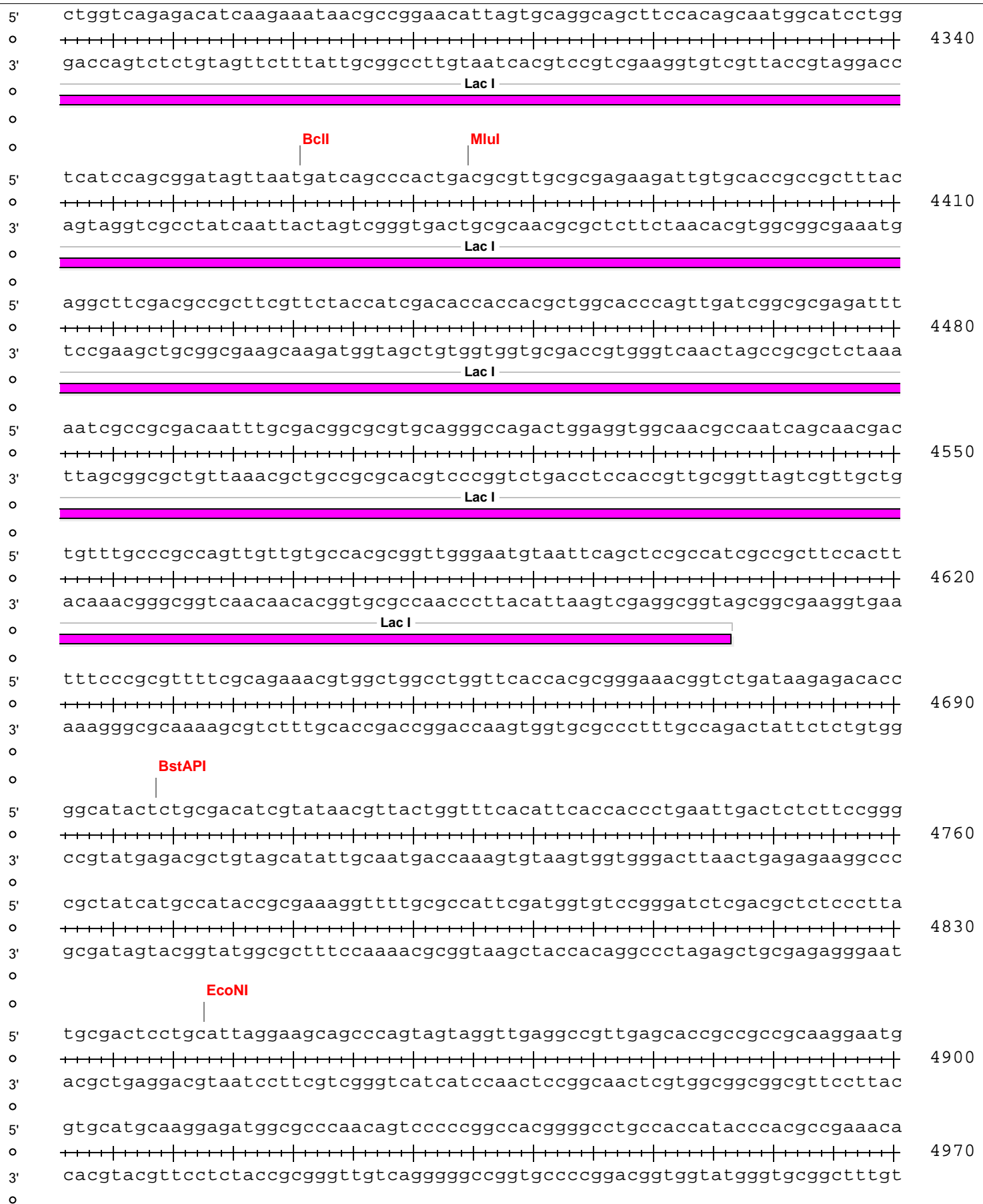
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3'	gccccgtacaattcccgcataaaaggacaaaccagtgactacggaggcacattccccctaaagacaagt	
o		
5'	tgggggtaatgataccgatgaaacgagagaggatgctcacgatacgggttactgatgatgaacatgcccg	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	2940
3'	acccccattactatggctactttgctctctctctacgagtgctatgcccaatgactactacttgtacgggc	
o		
5'	gttactggaacgttgtgagggtaaacactggcggtatggatgctggcgggaccagagaaaaatcactcag	
o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	3010
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o		
5'	ggtcaatgccagcgcttcggttaatacacagatgtaggtgtccacagggtagccagcagcatcctgcgatgc	
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o		
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o		
o		
	Bpu10I	
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	FspAI	
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o		
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o		
o		
	PshAI	
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o	++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++	3640
3'	caacttccgagagttcccgtagccagctctagggccacggattactcactcgattgaatgtaattaacgc	
o		
o		

B01a2-pET32-DEST



B01a2-pET32-DEST



B01a2-pET32-DEST

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 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' tcgcgagtactcgggcttcaccgctcgggctagaaggggtagccactacagccgctatatccgcggtcgt

SgrAI

5' accgcacctgtggcgccggtgatgccggccacgatgcgctccggcgtagaggatcgagatcgatctcgatc 5110
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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Xbal

5' ccgcgaaattaatacgactcactataggggaattgtgagcggataacaattcccctctagaataatttt 5180
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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T7 Promoter Lac Operator

BamI BamI'

5' gtttaactttaagaaggagatatacatatgagcgataaaattattcacctgactgacgacagttttgaca 5250
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' caaattgaaattcttcctctatatgtatactcgctattttaataagtggactgactgctgtcaaaactgt

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

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 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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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' gatcgccccgattctggatgaaatcgctgacgaatatcagggcaaactgaccggttgcaaaactgaacatc 5390
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
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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

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 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' ctagttttgggaccgtgacgaggctttataccgtaggcaccatagggctgagacgacgacaagtttttgc

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
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B01a2-pET32-DEST

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 0
 0
 Trx Tag

5530

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 0 ++++++
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 0
 0
 -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
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 0
 En...e

5670

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5740

Enterokinase attB1 TEV site
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 0 ++++++
 3' tccgTTTTGGAAATGGGTCTAAAGCATAACCCTCGTCGGTCAAGGCGGATAGTCCATCTTCCGCGGTGG
 0
 0
 T... BGH01_B01a2
 BGH01_B01a2-pDONR221

5810

G K T F N P D F V W G A A S S A Y Q V E G A T
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B01a2-pET32-DEST

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 3' TGGTGGCTACCGTCTCCGGGGTCATAAACCCCTACGCAAGTCAAGGTAAGGGCCATTTTGGATGGTGTGG

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————— **BGH01_B01a2-pDONR221** —————

T T D G R G P S I W D A F S S I P G K T Y H N
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5880

BmgBI

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————— **BGH01_B01a2** —————

————— **BGH01_B01a2-pDONR221** —————

Q N A D I A C D H Y N R W Q E D V A I M K E M G
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5950

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 3' CGATTTCCGAATGGCGAAAAGATAAAGAACCAGCGCGTATAAGGGATGACCCGCGCCGCTTCAATTGCTT

————— **BGH01_B01a2** —————

————— **BGH01_B01a2-pDONR221** —————

L K A Y R F S I S W S R I F P T G R G E V N E
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6020

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————— **BGH01_B01a2-pDONR221** —————

K G V A F Y N N L I D E L I K N D I T P W V T
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6090

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 3' ATAAAGTGACCCTGAAAGGAGACCGTGACGTTTACCTTTACCTGCGGATGAATTGGGGCGGTAGCGGCT

————— **BGH01_B01a2** —————

————— **BGH01_B01a2-pDONR221** —————

L F H W D F P L A L Q M E M D G L L N P A I A D
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6160

○
○
5' CGAATTCGCCAACTACGCCAAGCTGTGTTTCGCGCGCTTTGGCGACCGCGTTACCCACTGGATTACCCTA
○
3' GCTTAAGCGGTTGATGCGGTTTCGACACAAAGCGCGCAAACCGCTGGCGCAATGGGTGACCTAATGGGAT
○
————— **BGH01_B01a2** —————
○
————— **BGH01_B01a2-pDONR221** —————

6230

○
○
E F A N Y A K L C F A R F G D R V T H W I T L
○
319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341
○
5' AACGAACCTTGGTGCAGTGCCATGCTTGGCCACGGCATGGGCAGCAAAGCCCCTGGCCGCGTATCTAAGG
○
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○
————— **BGH01_B01a2** —————
○
————— **BGH01_B01a2-pDONR221** —————

6300

○
○
N E P W C S A M L G H G M G S K A P G R V S K
○
342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364
○
5' ATGAACCCTATATAGCCGCCACAACCTTGCTGCGTGACACGGCAAATGGTAGATATTTACCGGCGCGA
○
3' TACTTGGGATATATCGGCGGGTGTGTTGAACGACGCACGTGTGCCGTTTACCATCTATAAATGGCCGCGCT
○
————— **BGH01_B01a2** —————
○
————— **BGH01_B01a2-pDONR221** —————

6370

○
○
D E P Y I A A H N L L R A H G K M V D I Y R R E
○
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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○
3' TAAAGTCGGGTGTGTTTTTCCGTACTATCCGTATCGGTTGTTAACGCTGACCGCGCTTGGGTTTTGGCTA
○
————— **BGH01_B01a2** —————
○
————— **BGH01_B01a2-pDONR221** —————

6440

○
○
F Q P T Q K G M I G I A N N C D W R E P K T D
○
389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411
○
5' TCTGAATTAGATAAAAAAGCAGCCGAGCGGCCCTAGAATTTTTTGTAAAGCTGGTTTGCCGACCCCATTT
○
3' AGACTTAATCTATTTTTTTCGTCGGCTCGCGCGGGATCTTAAAAACATTTCGACCAAACGGCTGGGGTAAA
○
————— **BGH01_B01a2** —————
○
————— **BGH01_B01a2-pDONR221** —————

6510

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

B01a2-pET32-DEST

5' ATTTGGGCGACTACCCAGCCAGCATGCGCGAGCGCTTGGGTGAGCGTTTACCCACCTTTAGCGACGAAGA
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' TAAACCCGCTGATGGGTGCGTACGCGCTCGCAACCCACTCGCAAATGGGTGGAAATCGCTGCTTCT

----- BGH01_B01a2 -----
 ----- BGH01_B01a2-pDONR221 -----

6580

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

5' CATTGCGCTAATAAAAACTCTAGCGACTTTTTTGGTTTGAATCACTACACCACCATGCTTGCCGAACAA
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' GTAACGCGATTATTTTTTGAGATCGCTGAAAAAACCAAACCTTAGTGATGTGGTGGTACGAACGGCTTGT

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6650

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

AleI

5' ACCCACGAAGGTGACGTTGTTGAAGATACTATTCGCGGCAACGGCGGCATATCGGAAGACCAAATGGTCA
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' TGGGTGCTTCCACTGCAACAACCTTCTATGATAAGCGCCGTTGCCGCCGTATAGCCTTCTGGTTTACCAGT

----- BGH01_B01a2 -----
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6720

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

PasI

5' CCCTCTCAAAGACCCAAGCTGGGAACAAACCGACATGGAGTGGAGCATTGTGCCCTGGGGCTGTAAAAA
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' GGGAGAGGTTTCTGGGTTGACCCCTTGTGGCTGTACCTCACCTCGTAACACGGGACCCCGACATTTTT

----- BGH01_B01a2 -----
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6790

T L S K D P S W E Q T D M E W S I V P W G C K K
 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' ATTATTAATCTGGTTAAGCGAGCGCTACAACCTACCCGACATTTACATTACCGAAAACGGCTGCGCCCTA
 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
 3' TAATAATTAGACCAATTCGCTCGCGATGTTGATGGGGCTGTAAATGTAATGGCTTTTGCCGACGCGGGAT

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 ----- BGH01_B01a2-pDONR221 -----

6860

L L I W L S E R Y N Y P D I Y I T E N G C A L
 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551

0
5' C C C G A C G A A G A C G A C G T A A A C A T A G C C A T T A A C G A T A C A C G C C G C G T A G A T T T T T A C C G C G G T T A T A T C G
0
3' G G G C T G C T T C T G C T G C A T T T G T A T C G G T A A T T G C T A T G T G C G G C G C A T C T A A A A A T G G C G C C A A T A T A G C
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

6930

SacII

0
P D E D D V N I A I N D T R R V D F Y R G Y I
0
552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574
5' A T G C G T G T C A C C A A G C A A T A G A G G C C G G C G T A A A A C T A A A A G G C T A T T T T G C A T G G A C A C T T A T G G A T A A
0
3' T A C G C A C A G T G G T T C G T T A T C T C C G G C C G C A T T T T G A T T T T C C G A T A A A A C G T A C C T G T G A A T A C C T A T T
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

7000

0
D A C H Q A I E A G V K L K G Y F A W T L M D N
0
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' C T A C G A A T G G G A A G A A G G C T A C A C C A A A C G C T T T G G C T T A A A C C A T G T A G A T T T C A C C A C A G G C A A A C G C
0
3' G A T G C T T A C C C T T C T T C C G A T G T G G T T T G C G A A A C C G A A T T T G G T A C A T C T A A A G T G G T G T C C G T T T G C G
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

7070

0
Y E W E E G Y T K R F G L N H V D F T T G K R
0
599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621
5' A C A C C T A A A C A G T C T G C A A T T T G G T A T A G C A C G T T A A T T A A A G A T G G T G G G T T C T A G G A C C C A G C T T T C T
0
3' T G T G G A T T T G T C A G A C G T T A A A C C A T A T C G T G C A A T T A A T T T C T A C C A C C C A A G A T C C T G G G T C G A A A G A
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

7140

PacI

attB2

0
T P K Q S A I W Y S T L I K D G G F
0
622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640
5' T G T A C A A A G T G G T T G A T a t c g g a t c c g a a t t c g a g c t c c g t c g a c a a g c t t g c g g c c g c a c t c g a g c a c c
0
3' A C A T G T T T C A C C A A C T A t a g c c t a g g c t t a a g c t c g a g g c a g c t g t t c g a a c g c c g g c g t g a g c t c g t g g
————— **attB2** —————
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

7210

BamHI

EcoRI

SacI

Sall

HindIII

NotI

EagI

PspXI

XhoI

AvaI

BmeT110I

0
5' a c c a c c a c c a c c a c t g a g a t c c g g c t g c t a a c a a a g c c c g a a a g g a a g c t g a g t t g g c t g c t g c c a c c g c
0
3' t g g t g g t g g t g g t g a c t c t a g g c c g a c g a t t g t t t c g g g c t t t c c t t c g a c t c a a c c g a c g a c g g t g g c g
————— **BGH01_B01a2** —————
————— **BGH01_B01a2-pDONR221** —————

7280

o | **BipI**
5' tgagcaataactagcataacccttggggcctctaaacgggtcttgaggggttttttgcgaaaggagga
o +++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|+++|
3' actcgttattgatcgtattggggaaccccgagattgcccagaactccccaaaaaacgactttcctcct
o _____ T7 Terminator _____ ▀

7350

o
5' actatatccggat
o +++|+++|+++
3' tgatataggccta
o

7363