

o
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o
o
o

XbaI



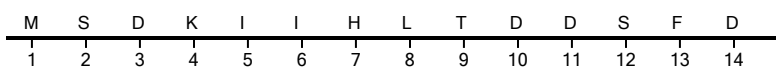
5180

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o
o

BamHI

BamHI'

Trx Tag

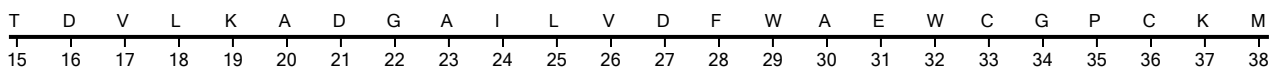


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RsrII

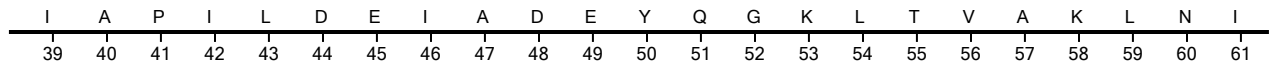
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5320

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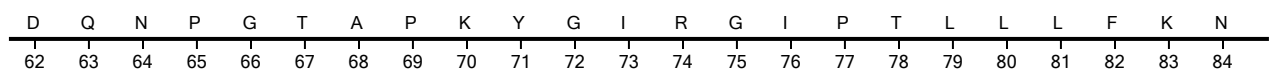
Trx Tag



5390

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o
o
o

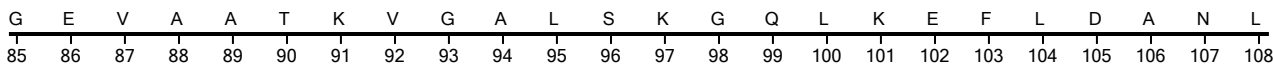
Trx Tag



5460

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3' cacttcaccgccggtggtttcacccacgtgacagatttccagtcactttctcaaggagctgcgatttga
o
o
o

Trx Tag



5530

BGT25_T02a3-pET32-DEST

5' ggccggttctgggttctggccatatgcaccatcatcatcatcattcttctgggtctgggtgccacgcggttct
 3' cggccaagaccaagaccgggtatacgtggtagtagtagtagtaagaagaccagaccacgggtgcgccaaga
 -T...g

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' ccatactttctttggcgacgacgatttaagcttgcggtcgtgtacctgctcgggtctagacctatggctgc

BgIII
Acc65I
KpnI

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

5670

5' acgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAAGCAGGCTctgaaaacttgtactttca
 3' tgctgctgttccgggtaccgctaTAGTTGTTCAAACATGTTTTTTCGTCCGAgacttttgaacatgaaagt

Enterokinase
attB1
TEV site

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5740

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' aggcGTCAATGAGCCGCCGAAACTTGGGGCGTTTCGGGACCGAGAGCTTGATCGACCAGCACAGGCTTGTC
 3' tccgCAGTTACTCGGCGGCTTGAACCCCGCAAGCCCTGGCTCTCGAACTAGCTGGTCTGTCCGAACAG

T...
procollagen-lysine 5-dioxygenase

BGT25_T02a3
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5810

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

5' CGGCAGCGGTTCGTTGCGGTCGCGCATCGAGGGCACGCCGCGCATCCTGGTGACAATCCTCGCAAAGCAGA
 3' GCCGTCGCCAGCAACGCCAGCGGTAGCTCCCGTGC GGCGCGTAGGACCACTGTTAGGAGCGTTTCGTCT

procollagen-lysine 5-dioxygenase

BGT25_T02a3
BGT25_T02a3-pDONR221

5880

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

BGT25_T02a3-pET32-DEST

5' AAGAGCCGGCACTGCCGCTGTACCTCGAGTGCATCGAGGCGCTCGACTACCCCAAGGCGTCGATCGTCCT
 3' TTCTCGGCCGTGACGGCGACATGGAGCTCACGTAGCTCCGCGAGCTGATGGGGTTCCGCAGCTAGCAGGA

5950

procollagen-lysine 5-dioxygenase

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K E P A L P L Y L E C I E A L D Y P K A S I V L
 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' GTACATCAGGACAAACAACAACACCGACAGGACCGAGCACATACTGCGCGAATGGGTGGAGCGCGTTGGT
 3' CATGTAGTCCTGTTTGTGTGGCTGTCCTGGCTCGTGTATGACGCGCTTACCCACCTCGCGCAACCA

6020

procollagen-lysine 5-dioxygenase

BGT25_T02a3

BGT25_T02a3-pDONR221

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

5' CACCTCTATGCCGCGGTTGAATTCGATGCCTCCAACGTGGCCGATAGAGTCGAGCAGTTTGGCGAACACG
 3' GTGGAGATACGGCGCCAACTTAAGCTACGGAGTTGCACCGGCTATCTCAGCTCGTCAAACCGCTTGTGC

6090

procollagen-lysine 5-dioxygenase

BGT25_T02a3

BGT25_T02a3-pDONR221

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

5' AATGGAACGAGACGCGCTTCAGGGTGCTTGCCGAATCCGCAACATAAGTTTGC GGAAAAC TCTCGAACA
 3' TTACCTTGCTCTGCGCGAAGTCCCACGAACCGGCTTAGGCGTTGTATTCAAACGCCTTTTGAGAGCTTGT

6160

procollagen-lysine 5-dioxygenase

BGT25_T02a3

BGT25_T02a3-pDONR221

E W N E T R F R V L G R I R N I S L R K T L E H
 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' CAGCTGCGATTTCTATTTTCGTTGCCGACGTCGATAATTTTCGTGCGACCGGCCACGCTACGCGAGCTTGT
 3' GTCGACGCTAAAGATAAAGCAACGGCTGCAGCTATTAAAGCACGCTGGCCGGTGCATGCGCTCGAACAG

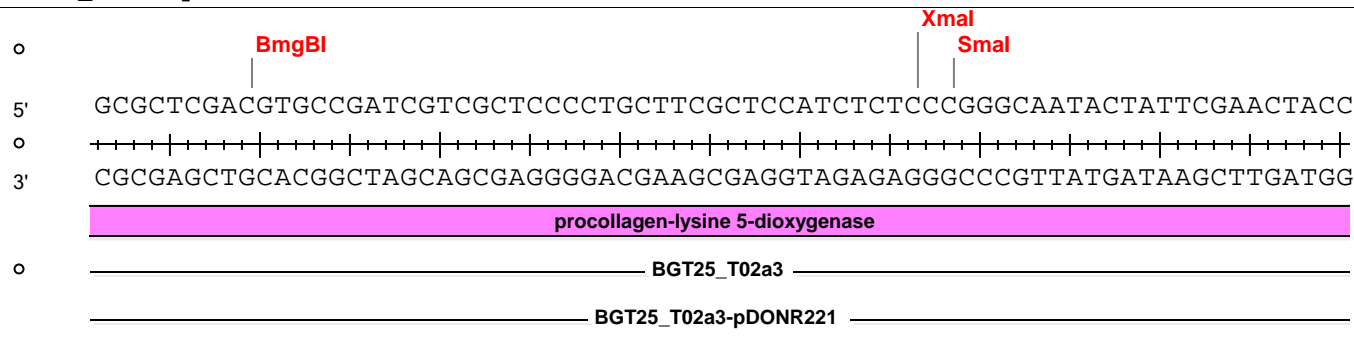
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procollagen-lysine 5-dioxygenase

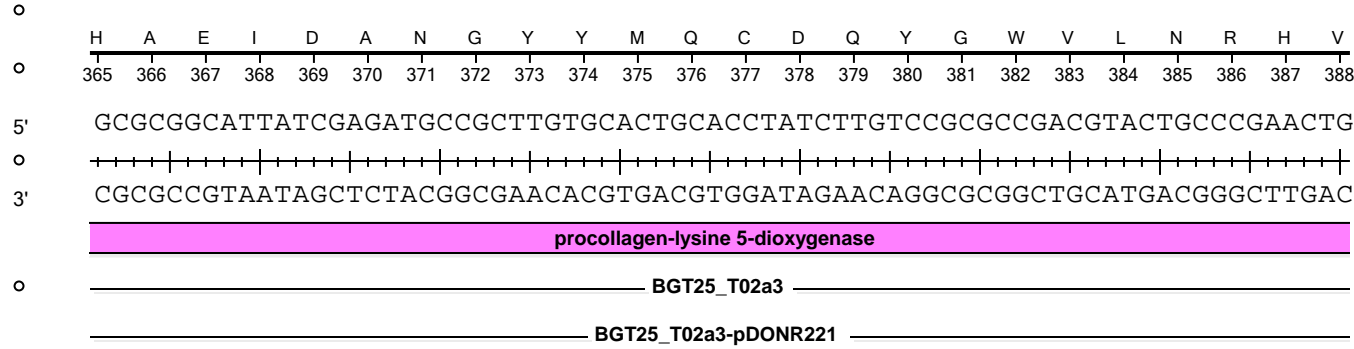
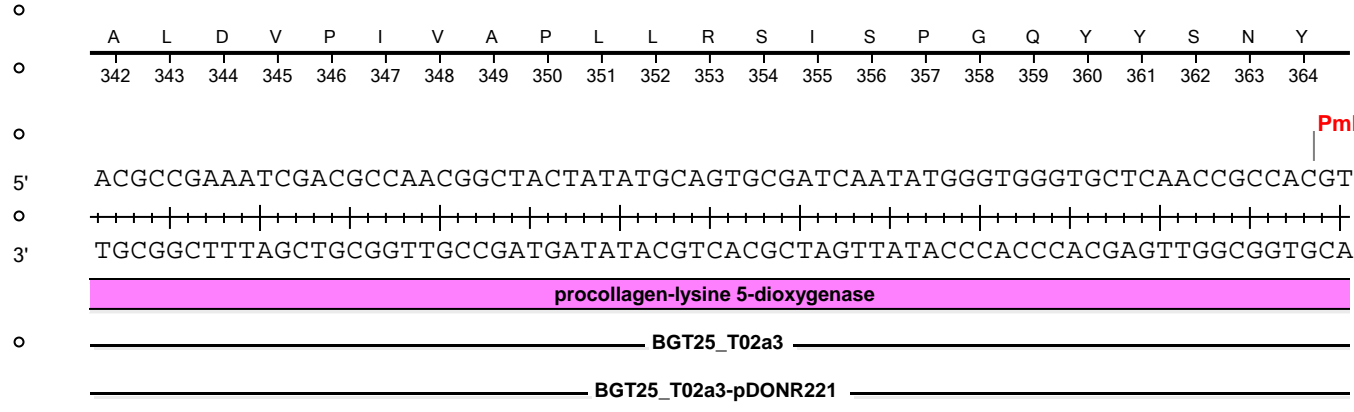
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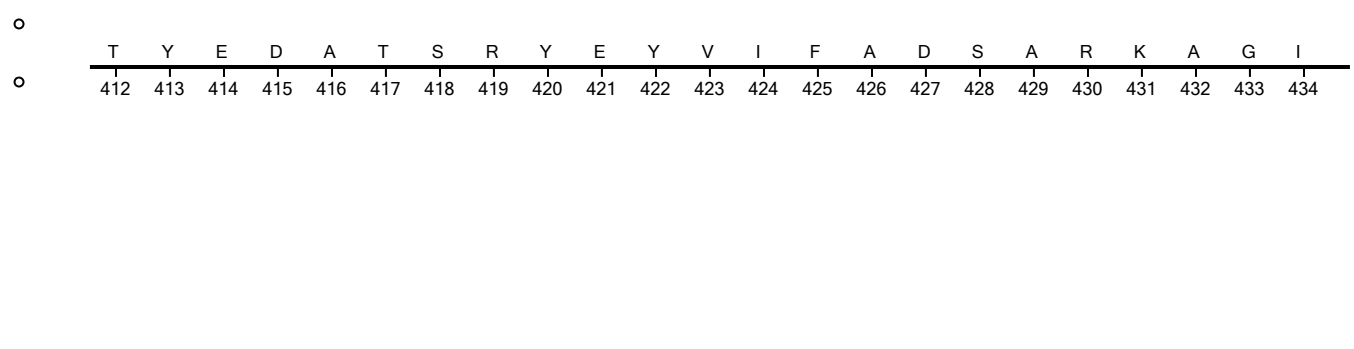
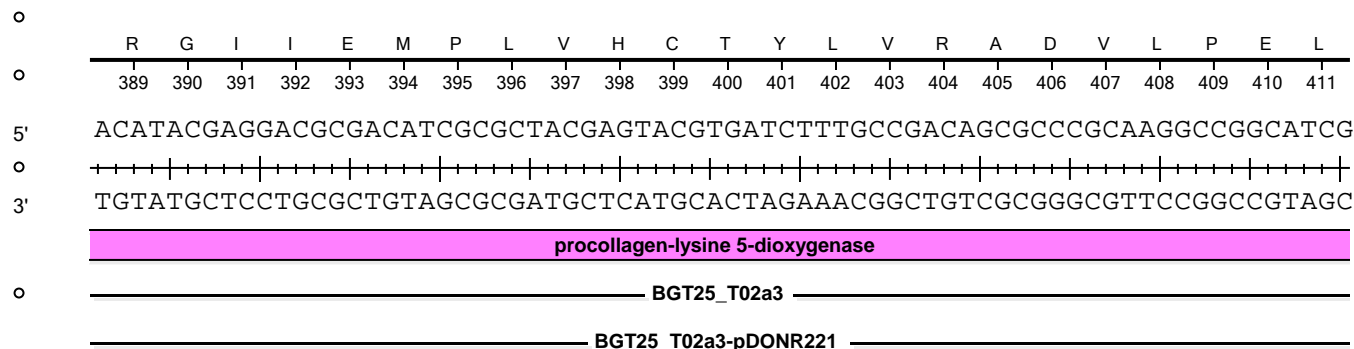
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 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341



6300



6370



6440



6510

BGT25_T02a3-pET32-DEST

5' TCCAGTATATGGATAACCGACAAGTCTATGGCTACATCACCTTCGGCGATGGCCAATATTATGTGAGTGA
 0 ++++++
 3' AGGTCATATACCTATTGGCTGTTTCAGATACCGATGTAGTGAAGCCGCTACCGGTTATAATACTCACT

6580

procollagen-lysine 5-dioxygenase

BGT25_T02a3

BGT25_T02a3-pDONR221

V Q Y M D N R Q V Y G Y I T F G D G Q Y Y V S 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' CGGGATCGAGCGCGCCCGAGCGCTGTTGCACGGTGC CGGCGACACGCCGGTATTCCTGCGCCACGCCT
 0 ++++++
 3' GCCCTAGCTCGCGCGGGCTCGCGACAACGTGCCACGGCCGCTGTGCGGCCATAAGTGACGGCGGTGCGGA

6650

pr...e

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GT25?

BGT25_T02a3-pDONR221

G I E R A R A L L H G A G D T P V F T A A T 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' CCGGGCGCCGTACCGATGCCAGATCGACATGTCGAGCCGCCGAAATTCACCTAATAAACTTGGACAGCA
 0 ++++++
 3' GGCCCGCGGCATGGCTACGGTCTAGCTGTACAGCTCGGCGGCGTTTAAGTGGATTATTTGAACCTGTCGT

6720

BGT25_T02a3

GT25?

BGT25_T02a3-pDONR221

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

5' GCGTCGAGCGCTGGCGCAAATATCAAGATAGGAATCCTCATCTAGCCGCCAATAACAATTCGGGTATCGGC
 0 ++++++
 3' CGCAGCTCGCGACCGCGTTTATAGTTCTATCCTTAGGAGTAGATCGGCGGTTATGTTAAGCCCATAGCCG

6790

BGT25_T02a3

GT25?

BGT25_T02a3-pDONR221

S V E R W R K Y Q D R N P H L A A N T I R V S A
 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528

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○

5' TGGTTGAACTTCGATTTCTCCAAAGCCAAGCTGGAGTTCTACAACCGACAACACAAACAGATTCCGGCCC

○

3' ACCAACTTGAAGCTAAAGAGGTTTCGGTTCGACCTCAAGATGTTGGCTGTTGTGTTTGTCTAAGGCCGGG

————— BGT25_T02a3 —————

7140

GT25?

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W L N F D F S K A K L E F Y N R Q H K Q I P A

622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644

○

5' AATTTCAATCTGAAACCTATCCGCGATCTCTTATCCGACTAGTCCATTCCTTTGGTGCAATGGCTTATTC

○

3' TTAAAGTTAGACTTTGGATAGGCGCTAGAGAATAGGCTGATCAGGTAAGGAAACCACGTTACCGAATAAG

————— BGT25_T02a3 —————

7210

Spel

GT25?

BGT25_T02a3-pDONR221

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○

Q F Q S E T Y P R S L I R L V H S F G A M A Y S

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' AGTAACTCCAAGGGGAGCTCAAGTACTTCTTGAAAAATGCCTACCTCTTCGAAAGCGGCTTATTCGGTTT

○

3' TCATTGAGGTTCCCCTCGAGTTCATGAAGAACTTTTACGGATGGAGAAGCTTTCGCCGAATAAGGCAAA

————— BGT25_T02a3 —————

7280

GT25?

BGT25_T02a3-pDONR221

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○

V T P R G A Q V L L E K C L P L R K R L I P F

669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691

○

5' CCTGGGACTGGAGTGGTCCTCGAGGATGAGGGCATCGACTGCGCAATGTCCGCGGTGTATGACTCGATGC

○

3' GGACCCTGACCTCACCAGGAGCTCCTACTCCCGTAGCTGACGCGTTACAGGCGCCACATACTGAGCTACG

————— BGT25_T02a3 —————

7350

GT25?

BGT25_T02a3-pDONR221

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○

P G T G V V L E D E G I D C A M S A V Y D S M

692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714

AbsI

BGT25_T02a3-pET32-DEST

o NsiI NruI

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o

3' TACGCAAGCAGACGTACGGCGGTGACCAGTAAGTGCTGCTGCTCGTCCGTAGTCTAGCCCTTCAGCGCTT

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GT25?

BGT25_T02a3-pDONR221

H A F V C M P P L V I H D D E Q A S D R E V A N

715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738

o BamHI HindIII

5' TCGGCAGTAGGACCCAGCTTCTTGTACAAAGTGGTTGATatcggatccgaattcgagctccgtcgacaa 7490

o

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BG...a3

attB2

GT25?

BGT25_T02a3-pDONR221

R Q .

739 740 741

o NotI

5' gcttgccggccgcactcgagcaccaccaccaccactgagatccggctgctaacaaagccgaaaggaa 7560

o

3' cgaacgccggcgtgagctcgtggtggtggtggtgactctaggccgacgattgtttcgggctttcctt

BplI

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T7 Terminator

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T7 Terminator