

pET32-DEST

5' tggcgaatgggacgcccctgtagcggcgcatgaagcggcggggtggtggttacgcgcagcgtgaccgctacacttgccagcgcctagcgcgccctcctttcgctttctcccttcttctcgcc
 130
 3' accgcttacctgcggggacatcgccgctaattcgccgccccacaccaccaatgcgcgtgcactggcgatgtgaacggtcgcgggatcgcgggcgagaaagcgaagaaggaaggaagagcgg

5' acgttcgcccgtttcccogtcaagctcctaatacgggggtccctttagggttccgatttagtgccttacggcacctcgacccccaaaaaacttgattagggtgatggttcacgtagtgggccatcgccct
 260
 3' tgcaagcggccgaaagggcagttcgagatttagccccgagggaaatccaaggctaaatcacgaaatgccgtggagctgggtttttgaaactaatcccactaccaagtgcaccccgtagcgga

5' gatagacgggtttttcgcccttgacgttggagtcacgttctttaatagtggaactctgttccaaactggaacaacactcaaccctatctcggctctattctttgattataagggattttgccgatttc
 390
 3' ctatctgcaaaaaagcggaaactgcaacctcaggtgcaagaaatatacactgagacaaggtttgacctgtgtgagttgggatagagccagataagaaaactaaatattccctaaaacggctaaag

5' ggccatttggttaaaaaatgagctgatttaacaaaaatataacgcaatataacgcttacaatttaggtggcacttttcgggaaatgtagcgggaacccctattgtttatTTTTCT
 520
 3' ccggataaccaatTTTTCTactgactaaattgttttaattgcttataattgcaatgttaaatccaccgtgaaaagccctttacacgcgccttggggataaacaataaaaaaga

5' aaatacatcaaatatgtatcgcctcatgagacaataaccctgataaatgcttcaataattgaaaaaggaagatagattcaacattccgtgtagccctattccctTTTTTGGCGATTGTG
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5' ccttctgtttttgctcaccagaacgctggtgaaagtaaagatgctgaagatcagttgggtgacagagtggttacatogaactggatctcaacagcggtaagatccttgagagttttcggccggaa
 780
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5' gaaagctttccaatgatgagcacttttaagttctgctatgtggcgcggtattatccogtattgacgcgggcaagagcaactcggctcgcgcatacactattctcagaatgacttggtgagtagctcac
 910
 3' cttgcaaaaggttactactcgtgaaaattcaagacgatacaccgcgcataataggcataactcggcccggttctcgttgagccagcggcgtatgtgataagagttctactgaaccaactcatgagtg

5' cagtcacagaaaagcatctacggatggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataaactcggccaacttactctgacaacgatcggaggaccgaaggagctaacccg
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5' agcaccgcctacatacctcgtctgtaactcctgttaccagtggtcgtcgcagtgccagtgccgataaagtctgtcttaccgggttgactcaagacgatagttaccggataaggcgcagcggctgggctgaacg
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5' gggggttctgacacagcccagcttgagcgaacgacctacaccgaactgagatacctacagcgtgagctatgagaaagcggcagcctcccgaaaggagaaaggcggacaggtatccggtaagcggca
 2080
 3' ccccaagacgctgtcgggtcgaacctcgttctgctgagtggttgaactctatggatgctgcactcagactccttccggtgcaagggcttccctctttccgctgctcataggccatttcgctg

AloI'

AloI

DrallI

PsiI

Ap

Ap

Ap

PvuI

Ap

Ap

Ap

AhdI

Ap

Ap

Ori

Ori

Ori

Ori

o

5' ataacgccgaacattagtgaggcagcttccacagcaatggcatcctggtcattccagcggatgtaataatgatcagccactgacgcgttgccgagaagattgtgaccgccgttttacaggcttgac 4420
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 3' tattgcgcccttgaatacacgctcgtgcaaggtgctggtaccgttaggaccagtaggtcgctatcaattactagtcgggtgactgcgcaacgcgctcttctaacacgtggcggaagtccgaagctg
 o
 Lac I

o

5' gccgcttcgttaccatogacaccaccagctggcaccagttgatcgcgcgagatttaatgcgcgcgacaatttgcagcgcgcgtgcaggccagactggaggtggcaacccaatcagcaacgac 4450
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 3' cggcgaagcaagatggttagctgtggtggtgacccgtgggtcaactagccgcgctctaaatagcggcgtgttaaacgctgcgcgcacgtcccggctcgacctccacggttgcggttagctgtgctg
 o
 Lac I

o

5' tgtttgcccgccagttgtgtgcccagcgggtgggaatgtaattcagctccgcatcgcgcgttccactttttcccgcttttcgcagaaacgtggctggcctggttaccacgcgggaaacggctgat 4480
 o
 3' acaaacggcggtcaacaacaggtgcccacccttacattaagtgcaggcgttagcggcgaagtgaaaaggcgcgcaaaagcgtctttgaccgcagccgcaagtggtgcccctttgccagacta
 o
 Lac I

o

BstAPI

5' aagagacaccggcactctgcgacatcgtataacgttactggtttcacattcaccaccctgaattgactctctccgggcgtatcatgccataccgcgaaaggtttgcccattcgatggtgtccgg 4810
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o

EcoNI

SphI

5' gatctcgacgctctcccttatgcgactcctgattaggaagcagccagtagtaggtgaggcgttgagcaccgccccgcaaggaatggtgcatgcaaggagatggcgcccaacagtccccggccac 4940
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o

SgrAI

5' gggcgctgccaccataccacgcgcaaacagcgtcattgagcccgaagtggcgagcccagctctcccatcgggtgatgctggcgatattagcgcagcaaccgacctgtggcgccgggtgatgccggcc 5070
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 3' ccccgacgctggtatgggtgcccgtttgttcgcgagtagctcgggcttcaccgcctcgggctagaaggggtagccactacagccgctatatcccggtgctgtggcggtgacaccgcggccactacggccgg

o

XbaI

BamHI

5' acgatcgcctccggcgtagaggatcgagatcgcgatcccgcaaatatacagactcaactataggggaattgtgagcggataacaattccctctagaataattttgtttaaactttaagaaggaga 5200
 o
 3' tgctacgagccgcatctcctagctctagctagagctaggcgcgttaattatgctgagtgatcccttaaacactcgcctattgttaagggagatctttatataaaacaaattgaaattcttctct

o

— T7 Promoter — — Lac Operator —

o

BamHI

RsrII

5' tatacatatgagcgataaaaattttacactgactgacgacagttttgacacggatgtaactcaaacgagcggggcgatcctcgtcgtattctgggcagagtggtgcccgtcggtaaaaatgatgccccc 5330
 o
 3' atatgtatactcgtatatttaataagtgactgactgctgcaaaactgtcctacatgagtttcgctgcccgcctagagcagctaaagaccgctctaccacgcagccagcagcttttactagcggggc

o

Trx Tag

M S D K I I H L T D D S F D T D V L K A D G A I L V D F W A E W C G P C K M I A P
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41

5' attctggatgaaatcgtcagcaaatatcagggcaaacgactgacgggtgcaaacgaaacatcgcatacaaacctggcactgcgcgcaaatatggatccggtggtatcccgactctgctgctgttcaaaaacg 5460
 o
 3' taagacctacttttagcagctgcttatagctccggttgactggcaacggtttgactgtgtagctagtttgggaccgtgacgcggctttataccgtaggcaccataggcgtgagacgacgacaagtttttgc

o

Trx Tag

I L D E I A D E Y Q G K L T V A K L N I D Q N P G T A P K Y G I R G I P T L L L F K N
 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84

5' gtgaagtggcggcaacaaagtggtgcaactgtcctaaggtcagtgaaagagtctcgcagcgtaacctggccggttctggtctggccatagcaccatcatcatcattctctggtctggtgcc 5590
 o
 3' cacttaccgcggcttgggttaccaccgctgacagatttccagtcactttctcaaggagctgcgattggaccggccaaagaccagccggtatagctggttagtagtagtaagaagaccagaccagcgg

o

Trx Tag

His6-tag

Thrombin

G E V A A T K V G A L S K G Q L K E F L D A N L A G S G S G H M H H H H H S S G L V P
 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128

o

BstBI

Acc65I

KpnI

5' acgcggttctggtatgaaagaaaccgctgctgtaaaattcgaaccgagcacatggacagccagatctgggtaccgacgacgacgacaaggccatggcgatATCAACAAGTTTGTACAAAAAGCTGAA 5720
 o
 3' tgcgcaagaccatacttctttggcagcagcatttaagcttgcggtcgtgtaacctgtcgggtctagaccatggctgctgctgctgctccggtagccgtaTAGTTGTTCAAACATGTTTTTCGACTT

o

Thrombin

Enterokinase

attR1

R G S G M K E T A A A K F E R Q H M D S P D L G T D D D D K A M A I S T S L Y K K A E
 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171

5' CGAGAACGTAATAATGATATAAATCAATATATAAATTAGATTTTGCATAAAAAACAGACTACATAAATCTGTAATAAACACACATATCCAGTCACTATGGCGGCCGATTAGGCACCCAGGCTTTAC 5850
 o
 3' GCTCTTGCATTTTACTATATTTATAGTTATATAAATTAATCTAAAACGATTTTTTGTCTGATGATATTATGACATTTTGTGTTGTATAGGTCAGTGATACCCGCGCGTAAATCCGTTGGGTCGGAATG

o

attR1

R E T
 172 173 174 175

5' ACTTTATGCTTCCGGCTCGTATAATGTGTGATTTTGAAGTTAGGATCCGTCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCAT 5980
 o
 3' TGAAAATACGAAGCCGAGCATATTACACACCTAAAACCTCAATCCTAGGCAGCTCTAAAAGTCCGATTCCTTCGATTTTACCTCTTTTTTTAGTGACCTATATGGTGGCAACTATATAGGGTTACCGTA

o

CM

pET32-DEST

5' CGTAAAGAACATTTTGGAGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTTTCAGTCGGATATTACGGCCTTTTAAAGACCGTAAAGAAAATAAGCACAAGTTTATCCGGCCTTTATTC
 0' ++++++
 3' GCATTTCTTGTAAACTCCGTAAAGTCAGTCAACGAGTTACATGGATATTGGTCTGGCAAGTCGACCTATAATGCCGAAAAATTTCTGGCATTCTTTTATTCGGTTCAAAATAGCCCGAAATAAG
 0' ++++++
 CM

5' ACATTTCTGCCCCCTGATGAATGCTCATCCGGAATCCGTATGGCAATGAAAGACGGTGGTGGATATGGGATAGTGTACCCCTTGTACACCGTTTCCATGAGCAAACGAAACGTTTTCATC
 0' ++++++
 3' TGTAAGAACGGCGGACTACTTACGAGTAGGCCCTTAAGGCATACCGTTACTTCTGCCACTCGACCATAACCCATCACAAGTGGGAACAATGTGGCAAAGGACTCGTTTGACTTTGCAAAAGTAG
 0' ++++++
 CM

5' GCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTACGGTGAACCTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTCTGCTCAGCC
 0' ++++++
 3' CGAGACCTCACTTATGGTGTCTAAAGGCCGTCAAAGATGTGTATATAAGCGTCTACACCGCACAATGCCACTTTTGGACCGGATAAAGGGATTCCCAAATAACTCTTATACAAAAAGCAGAGTCGG
 0' ++++++
 CM

5' **PasI**
 AAATCCCTGGGTGAGTTTACCAGTTTTGATTTAAACGTGGCCAATATGGACAACCTTCTCGCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGGCACAAGGTGCTGATGCCCTGGCGATTACAGG
 0' ++++++
 3' TTAGGGACCCACTCAAAGTGGTCAAACTAAATTTGCACCGGTTTACCTGTTGAAGAAGCGGGGCAAAAGTGGTACCCTTTATAATATGCGTTCCTGTTCCACGACTACGGCGACCGCTAAGTCC
 0' ++++++
 CM

5' TTCATCATGCCGTTTGTGATGGCTTCCATGTCGCGAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGCGTAAAGATCTGGATCCGGCTTACTAAAAGCCAGATAACAGTATGC
 0' ++++++
 3' AAGTAGTACGGCAAACACTACCGAAGGTACAGCGCTTACGAATTAATGTTGTCATGACGCTACTCACCGTCCCGCCCGCATTTCAGACTAGGCCAATGATTTTCGGTCTATTGTCATACG
 0' ++++++
 CM

5' GTATTTGCGCGCTGATTTTGGCGTATAAGAATATATACTGATATGTATACCCGAAGTATGTCAAAAAGAGGTATGCTATGAAGCAGCGTATTACAGTGACAGTTGACAGCGACAGCTATCAGTTGCTCA
 0' ++++++
 3' CATAAACCGCGACTAAAACGCCATATTCTTATATATGACTATACATATGGGCTTCATACAGTTTTTCTCCATACGATACTTCGTCGCATAATGTCACGTGCACTGTCGCTGTCGATAGTCAACGAGT
 0' ++++++
 BbvCI

5' AGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAACCATGCAGAATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTTATTGAA
 0' ++++++
 3' TCCGTATATACTACAGTTATAGAGCCAGACCAATTCGTGTTGGTACGCTTACTTCGGGCAGCAGACGCGCTTGGCAGCTTTCGCCCTTTAGTCTTCCCTACCGACTCCAGCGGGCCAAATAACTT
 0' ++++++
 XmaI
 SrfI
 SmaI

5' ATGAACGGCTCTTTTGTGACGAGAACAGGGGCTGGTGAATGCAGTTTAAAGTTTACACCTATAAAAGAGAGACCGGTTATCGTCTGTTTGTGGATGTACAGAGTATATTATGACACGCCCGGGCGA
 0' ++++++
 3' TACTTGCCGAGAAAACGACTGCTCTTGTCCCGACCACTTACGTCAAATCCAAATGGGATATTTCTCTCGGCAATAGCAGACAAACCTACATGTCTCACTATAATAACTGTGCGGGCCCGCT
 0' ++++++
 ccdB

5' CGGATGGTATCCCCCTGGCCAGTGCACGCTGCTGTCAGATAAAGTCTCCCGTGAACCTTACCCGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTCCGGTCT
 0' ++++++
 3' GCCTACCCTAGGGGGACCGGTACGTCAGACGACAGTCTATTTTTCAGAGGGCACTTGAATGGGCCACCAGTATAGCCCTACTTTCGACCGCGTACTACTGGTGGCTATACCGGTACACGGCCAGA
 0' ++++++
 ccdB

5' CCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGCGAAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGGAATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGTTCGACCATA
 0' ++++++
 3' GGCAATAGCCCTTCTTACCAGCTAGAGTCGGTGGCGCTTTTACTGTAGTTTTTTCGGTAATGGACTACAAGACCCCTTATATTTACAGTCCGAGGGAATATGTCGGTTCAGACGCTCCAGCTGGTAT
 0' ++++++
 ccdB at...2

5' GTGACTGGATATGTTGTGTTTTACAGTATTATGTAGTCTGTTTTTATGCAAAATCTAATTTAATATATGATATTTATATCATTTTACGTTTCTCGTTCAGCTTCTTGTACAAAGTGGTGTGATatcgg
 0' ++++++
 3' CACTGACCTATAACAACAAAATGTCATAATACATCAGACAAAAATACGTTTTAGATTAATATATAACTATAAATATAGTAAAAATGCAAAGGCAAGTCGAAAGAACATGTTTACCAACTAtagcc
 0' ++++++
 attR2

5' atccgaattcgagctccgtcgacaagcttgcgcccactcgagcaccaccaccaccactgagatccggtgctaacaaagcccgaaggaagctgagttggtgctgocaccgctgagcaataact
 0' ++++++
 3' taggcttaagctcgaggcagctgttcgaacccggtgagctcggtggtggtggtgactctaggccgacgattgtttcgggcttcccttcgactcaaccgacgagctggcgactcgttattga
 0' ++++++
 T7 Terminator

5' agcataacccttggggcctctaaccgggtcttgaggggtttttgctgaaaggaggaactatataccggt
 0' ++++++
 3' tcgtattggggaaccccgagatttgcccagaactccccaaaaacgactttctccttgatagggccta
 0' ++++++
 T7 Terminator