

T7 promoter primer →

BglII lac operator
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCC

XbaI rbs NdeI
CCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGACTATGACAAGACTGAAGATT
M T M T R L K I

TCGAAAACCTCTGCTGGCTGTAATGTTGACCTCTGCCGTCGCGACCGGCTCTGCCTACGCGGAAAAACAAC
S K T L L A V M L T S A V A T G S A Y A E N N

GCGCAGACTACCAATGAAAGCGCAGGGCAAAAAGTCGATAGCTCTATGAATAAAGTCGGTAATTTTCATG
A Q T T N E S A G Q K V D S S M N K V G N F M

GATGACAGCGCCATCACCGCGAAAAGTGAAGGCGGCCCTGGTGGATCATGACAACATCAAGAGCACCGAT
D D S A I T A K V K A A L V D H D N I K S T D

ATCTCTGTA AAAACCGATCAAAAAGTCGTGACCCTGAGCGGTTTCGTTGAAAGCCAGGCCAGGCCGAA
I S V K T D Q K V V T L S G F V E S Q A Q A E

GAGGCAGTGAAAGTGGCGAAAAGCGTTGAAGGGGTGACCTCTGTGACGCGACAAACTGCACGTTTCGCGAC
E A V K V A K G V E G V T S V S D K L H V R D

GCTAAAGAAGGCTCGGTGAAGGGCTACGCGGGTGACACCGCCACCACCAGTGAAATCAAAGCCAAAAC TG
A K E G S V K G Y A G D T A T T S E I K A K L

CTGGCGGACGATATCGTCCCTTCCCGTCATGTGAAAGTTGAAACCACCGACGGCGTGGTTCAGTCTCC
L A D D I V P S R H V K V E T T D G V V Q L S

GGTACCGTCGATTCTCAGGCACAAAAGTGACCGTGCTGAAAGTATCGCCAAAGCGGTAGATGGTGTGAAA
G T V D S Q A Q S D R A E S I A K A V D G V K

AGCGTTAAAAATGATCTGAAAAC TAAGATGGGTACCACCACCACCACCACCACCACCACCACCGGTAGC
S V K N D L K T K M G H H H H H H H H H H G S

CTGCAAGATTCGGAAGTCAACCAAGAAGCAAAGCCGGAAGTCAAGCCGGAAGTCAAACCGGAAACCCAT
L Q D S E V N Q E A K P E V K P E V K P E T H

ATTAACCTGAAAGTTAGTGACGGCAGCTCTGAAATTTTCTTTAAGATCAAAAAGACCACGCCGCTGCGT
I N L K V S D G S S E I F F K I K K T T P L R

CGCCTGATGGAAGCGTTTGCCAAAACGTCAGGGCAAGGAAATGGATAGCCTGCGTTTCCTGTATGACGGT
R L M E A F A K R Q G K E M D S L R F L Y D G

→
ATTTCGATCCAGGCAGATCAAGCGCCGGAAGACCTGGACATGGAAGACAACGACATCATTGAAGCCAC
I R I Q A D Q A P E D L D M E D N D I I E A H

XhoI BamHI
CGTGAACAGATTGGCGGC TCGAGGATCCGGCTGCTAACAAAGCCCCGAAAGGAAGCTGAGTTGGCTGCTG
R E Q I G G

BlpI T7 terminator
CCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAACGGGTCTTGAGGGGTTTTTTTG

← T7 terminator primer

The DNA sequence the of the *Escherichia coli* secreted *osmY* gene is shown in green, and the *Saccharomyces cerevisiae* SUMO protein Smt3 is shown in blue. The native *E. coli* protein OsmY possesses a highly efficient secretion signal at the amino terminus, and robust protein expression in the periplasm somehow causes protein to be excreted from cells into the media. The *S. cerevisiae* SUMO protease Ulp1 will cleave after the final Gly codon at the end of Smt3, leaving your protein completely unlabeled. Note: Ulp1 cannot cut if the residue immediately downstream is a proline (a.k.a. the first residue of your protein).

To insert genes by Gibson assembly, first cut the vector with *XhoI*. This will leave the 3' end of the coding strand precisely at the end of the *smt3* gene, making it easy to insert your protein sequence directly in-frame. Don't forget to include a stop codon at the end of your gene as this vector does not include one.

This vector carries a kanamycin resistance cassette, and was built by replacing the MCS of pET29b+

The *XhoI* restriction endonuclease cleaves at:

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5' - C|T C G A G -3'
3' - G A G C T|C -5'
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For Gibson cloning, include the following cassettes at the ends of the coding strand of your construct:

upstream cassette

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5' - CCGTGAACAGATTGGCGGC -3'
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downstream cassette (with two stop codons)

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5' - TAATGAGGATCCGGCTGCTAACAAAG -3'
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The amino acid sequence of the mature fusion protein is:

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ENNAQTTNESAGQKVDSSMNKVGNFMDDSAITAKVKAALVDHDNIKSTDISVKTDQKVVTLSGFVESQAQAEAVKVAK
GVEGVTSVSDKLHVRDAKEGSVKG YAGDTATTSEIKAKLLADDIVPSRHVKVETTDG VVQLSGTVDSQAQSDRAESIAK
AVDGVKSVKNDLKT KMGHHHHHHHHHGS LQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLME
AFAKRQ GKEMDSL RFLYDGIRIQADQAPEDLDMEDNDIIEAHREQIGG
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