

T7 promoter primer →

BglIII lac operator
 AGATCTCGATCCCGCAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCC

XbaI rbs NdeI
 CCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGGTCACCACCACCACCAC
M G H H H H H

CACCACCACCACCACGGTAGCCTGCAAGATTCGGAAGTCAACCAAGAAGCAAAGCCGGAAGTCAAG
 H H H H H G S L Q D S E V N Q E A K P E V K

CCGGAAGTGAACCCGAAACCCATATTAACCTGAAAGTTAGTGACGGCAGCTCTGAAATTTTCTTT
 P E V K P E T H I N L K V S D G S S E I F F

AAGATCAAAAAGACCACGCCGCTGCGTCGCTGATGGAAGCGTTTGCCAAACGTCAGGGCAAGGAA
 K I K K T T P L R R L M E A F A K R Q G K E

primer 316 →

ATGGATAGCCTGCGTTTCTGTATGACGGTATTCGCATCCAGGCAGATCAAGCGCCGGAAGACCTG
 M D S L R F L Y D G I R I Q A D Q A P E D L

XhoI BamHI

GACATGGAAGACAACGACATCATTTGAAGCCCACCGTGAACAGATTGGCGGCTCGAGGATCCGGCTG
 D M E D N D I I E A H R E Q I G G

BlpI T7 terminator

CTAACAAAGCCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCC
 ← T7 terminator primer

CTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTG

The DNA sequence of the *Saccharomyces cerevisiae* SUMO protein Smt3 is shown in [blue](#).

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 an ampicillin resistance cassette. This vector also contains a yeast origin of replication and uracil synthesis cassette for growth in yeast and selection using Ura-dropout media. This allows for cloning by yeast recombineering methods.

The *XhoI* restriction endonuclease cleaves at:

5' - C|T C G A G -3'
 3' - G A G C T|C -5'

For Gibson cloning, include the following cassettes at the ends of the coding strand of your construct:

upstream cassette

5' - CCGTGAACAGATTGGCGGC -3'

downstream cassette (with two **stop codons**)

5' - **TAATG**AGGATCCGGCTGCTAACAAAG -3'