

T7 promotor primer →

BglII lac operator
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAATTGTGAGCGGATAACAATTCC

XbaI rbs NdeI
CCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGGTCACCATCATCACCAC
M G H H H H H

CATCACCATCACCACGGTAGCGCGGCAATTCAACAAACGTTAGCCAAAATGGGCATCAAAAGCAGC
H H H H H G S A A I Q Q T L A K M G I K S S

GATATTTCAGCCCGCGCCCGTAGCTGGCATGAAGACAGTTCTGACTAACAGCGGCGTGTGTACATC
D I Q P A P V A G M K T V L T N S G V L Y I

ACCGATGATGGTAAACATATCATTCAGGGGCCAATGTATGACGTTAGCGGCACGGCTCCGGTCAAT
T D D G K H I I Q G P M Y D V S G T A P V N

GTCACCAATAAGATGCTGTTAAAGCAGTTGAATGCGCTTGAAAAAGAGATGATCGTTTATAAAGCG
V T N K M L L K Q L N A L E K E M I V Y K A

CCGCAGGAAAAACACGTCATCACCGTGTCTTACTGATATTACCTGTGGTTACTGCCACAACTGCAT
P Q E K H V I T V F T D I T C G Y C H K L H

GAGCAAATGGCAGACTATAACGCGCTGGGGATCACCGTGCCTTATCTTGCTTTCCCGCGCCAGGGG
E Q M A D Y N A L G I T V R Y L A F P R Q G

CTGGACAGCGATGCAGAGAAAAGAAATGAAAGCTATCTGGTGTGCGAAAGATAAAAAACAAAGCGTTT
L D S D A E K E M K A I W C A K D K N K A F

GATGATGTGATGGCAGGTAAAAGCGTCGCACCGAGCCAGCTGCGACGTGGATATTGCCGACCATTAC
D D V M A G K S V A P A S C D V D I A D H Y

GTACTTGGCGTCCAGCTTGGCGTTAGCGGTACTCCGGCAGTTGTGCTGAGCAATGGCACACTTGTT
V L G V Q L G V S G T P A V V L S N G T L V

CCGGGTTACCAGCCGCCGAAAGAGATGAAAGAATTCTCTGACGAACACCAAAAAATGACCAGCGGT
P G Y Q P P K E M K E F L D E H Q K M T S G

BamHI
AAAGGTAGCGAAAACCTGTATTTTCAGGATCCGGCTGCTAACAAAGCCCCGAAAGGAAGCTGAGTTG
K G S E N L Y F Q

BspI T7 terminator
GCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGT
← T7 terminator primer

TTTTTG

The DNA sequence of the N-terminal 10-His tag is shown in **blue**, the disulfide isomerase *dsbC* is shown in **green**, and the TEV protease recognition sequence is shown in **red**. Please note, DsbC is a dimer.

To insert genes by Gibson assembly, first cut the vector with *Bam*HI. This will leave the 3' end of the coding strand precisely at the end of the TEV recognition peptide, 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.

Note: The cystine-nucleophile protease **TEV** cuts at the C-terminal side of its recognition sequence "**ExxYxQ**" leaving your protein unlabeled. However, it cannot cut if either of the next two residues immediately downstream is a proline (a.k.a. the first or second residue of your protein).

This vector carries a kanamycin resistance cassette, and the vector was created by modifying pET29b.

The *Bam*HI restriction endonuclease cleaves at:

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

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

upstream cassette

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5' - GCGAAAACCTGTATTTTCAG - 3'
```

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

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5' - TAATGACGGCTGCTAACAAAGCCCGA - 3'
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