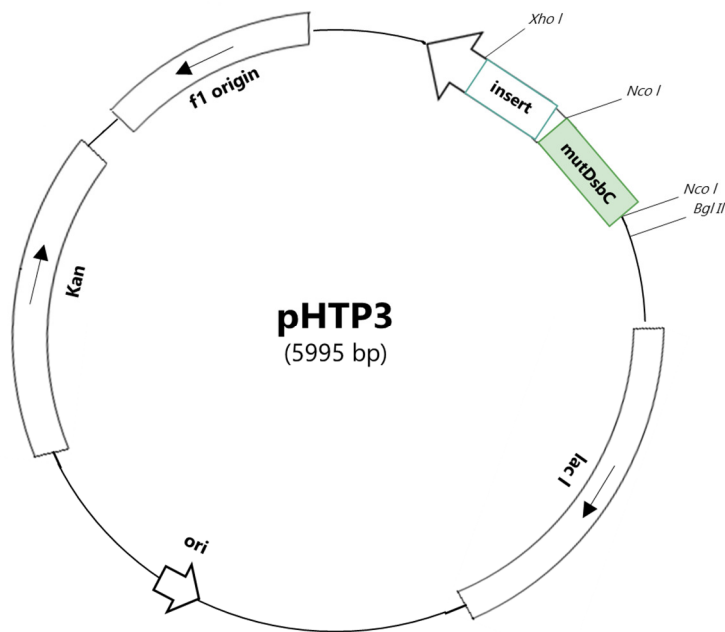


pHTP3 Expression Vector

pHTP3 was designed for the cloning and expression of high-levels of recombinant proteins in *Escherichia coli*. Recombinant proteins are expressed in fusion with an inactive form of disulfide-bond isomerase (mutDsbC), which is able to promote solubility and folding of disulfide bond-containing partners in the cytoplasm. This vector, included in the portfolio of NZYTech pHTP expression vectors, is part of the NZYEasy Cloning & Expression System. pHTP3 contains two poly-histidine (6xHis) sequences (N- and C-terminal) which allow subsequent recombinant protein purification by immobilized metal ion affinity chromatography (IMAC).

1. Vector Map



pHTP3 Cloning/Expression Region

<i>Nco I</i>	LLmutDsbC	<i>Nco I</i>	His-Tag
<u>CCATGG</u> GAGATGACGCGGCAATTCACAAACG. 648bp. AAAATGACCAGCGGTAAAGGATCAT		<u>CCATGG</u> GCAGCAGCCATCATCATCATCATCACAGCAGCGGC	
MetGlyAspAspAlaAlaIleGlnGlnThr. 216aa. LysMetThrSerGlyLysGlySerSerMetGlySerSer		HisHisHisHisHisHisHisSerSerGly	
CCTCAGCAAGGGCTGAGG / ⚡ / CCTCAGCTCCGCTGAGGTCGTCGACAAGCTTGCGGCCGCA		<i>Xho I</i>	His-Tag
ProGlnGlnGlyLeuArg / ⚡ / ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaLeuGlu		<u>CTCGAGCACCACCACCACCACCAC</u>	<i>STOP</i>
		HisHisHisHisHisHisHis*	

⚡ Represents the site where the gene will be inserted.

Note: For correct expression, inserted gene needs to be in frame with pHTP3 5' gene sequence. Inserts correctly cloned into pHTP3 will maintain reading frames starting on the ATG codon.

2. Vector Sequence (5995 bp)

TGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTCCCT
 TCCTTTCTCGCCACGTTTCGCGCGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTAGGGTTCGATTAGTGTCTTACGGCACCTCGACCCAAAAAACTTGATTAGGGTGATG
 GTTCACGATAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTGGACGTTGGAGTCCAGCTTCTTAATAGTGGACTCTTGTCCAACTGGAAACACACTCAACCCATCTCGGT
 CTATTTCTTTGATTATAAGGGATTTTGGCGATTTCGGCCTATTGGTTAAAAATGAGCTGATTTAACAAAAATTTAACCGGAATTTAACAAAAATATAACGTTTACAATTTAGGT
 GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTGTTTATTTCCTAAATACATCAAAATATGTATCCGCTCATGAATTAATCTTAGAAAACTCATCGAGCATCAAAATGAAAC
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 CGATTCGAGCTTCGCAACATCAATAACCACTTAAATTTCCCTCGTCAAAAAAAGGTTTAACTCAAGTGAGAAATCACCATGAGTGACGACTGAAATCCGGTGAGAAATGGCAAAAAGTTT
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 TCGCTGTTAAAAGGACAATTACAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTAATACCTGGAATGCTG
 TTTTCCCGGGATCGCAGTGGTGAATGCATCATCAGGAGTACGGATAAAAATGCTTGTATGGTGGAAAGAGGCATAAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGT
 AACATTTTGCACACATCTTCTTTCGCGTTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTTAATCCGCTTTAGTGAGCTGATACCGCTCGCCGACCCGAAACCGAGCCATT
 TACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTTATTACTGTTATGTAAGCAGACAGTTT
 TTGTTATGACAAAAATCCCTTAACTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAAGATCAAAAGGATCTTCTTGAATCCTTTTTTCTGCGCGTAACTGCTGCTGTC
 AAAAAAAAAACCAGCTACCGCGGTGGTTTGTTCGCGGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTACGAGAGCGCAGATACCAAACTACTGTCTTCTAGT
 GTAGCTGATTTGCTTTCGCTTTCGCGGTTTACCCGCTTTCGCGGATTAACCGCTTTAGTGAGCTGATACCGCTCGCCGACCCGAAACCGAGCCGAAACCGAGCCGAGCG
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 CAGACAAGCTGTGACCGTCTCCGGGAGCTGATGTGTCAGAGTTTTTACCCTCATACCGAAACCGCGCAGGCGAGCTGCGGTAAGGCTCATCAGCGTGGTCTGAAGCGATTACAG
 ATGCTGCGCTGTTTCATCCCGCTCCAGCTCGTTGAGTTTCTCCAGAAGCGTTAATGTCTGGTCTGATAAAGCGGCGCATGTTAAGGCGGTTTTTCTGTTTGGTCTAGTACGCT
 CCGTGAAGGGGATTTCTGTTTACGGGGTAATGATACCGATGAAACGAGAGAGGATGCTCACGATACCGGTTACTGATGATGAACATCGCCGTTACTGGAACGTTGTGAGGCTAA
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 ATCCGGAACATAATGTTGTCAGGCGCTGACTTCCGCTTTCAGACTTTACGAAACAGGAAACCGAAGACCATTATGTTGTTGCTCAGTTCGAGACGTTTTCAGCAGCAGTCGCT
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 GCGATAAATGGCTGCTTCTCGCGAAACGTTTGGTGGCGGACAGTGAAGAGGCTTGGAGGCGGTTGAGCGAGGCGTGAAGATTCCGAATACCGCAAGCACAGGCGCATCTCGTCCGCTC
 CAGCGAAAGCGGCTCTCGCGAAATGACCCAGAGCGCTGCCGCGCTGTCTACGAGTTGCATGATAAAGAAGCAGTCATAAGTGCAGCAGCAGATAGTCATGCCCGCGCCACC
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 CCTGCTGCCAGCTGCATTAATGAATCGGCCAACCGCGGGGAGAGGCGGTTTGGCTATTTGGGCGCAGGTTGGTTTTCTTTTACCAGTGAGAGGGGCAACAGCTGATTTGCCCTT
 CACCGCTTGGCTGAGAGATTCAGCAAGCGGTTCCACGCTGGTTTTGCGCCAGAGCGGAAATCCTGTTTATGTTGGTAAACGGCGGATATAACATGAGTGTCTTCGGTATCG
 TCGTATCCACTACCGAGATATCCGACCAACCGCGCAGCCGACTCGGTAATGGCGCATTTGCCCCAGCGCATCTGATCGTTGGCAACCAGCATCGCAGTGGGAACGATGCCCT
 CATTACGATTTGCATGGTTTGTGAAAACCGGACATGGCACTCCAGTCGCTTCCCGTTCGCTATCGGCTGAATTTGATGCGAGTGAGATATTTATGCCAGCCAGCCAGACGCG
 ACGCGCCGAGACAGAATTAATGGGCGGCTAACAGCGGATTTGCTGGTGACCAATGCGACAGATGCTCCACGCCAGTTCGCTACCTCTTCATGGGAGAAAAATAACTGTTG
 ATGGGTTCTGCTCAGACATCAAGAAATTAACCGCGGAACTAGTGACAGGCAATTCACAGCAATGCGATCCTGTCATCAAGCGGATGTTAATGATCAGCCACTGACCGCTT
 GCGCGAGAAGATTGTGACCCGCGCTTTACAGGCTTCGACGCGCTTCGTTTACCATCGACACCACCGCTGGCACCCAGTTGATCGCGCGAGATTAAATCGCCGCGACAATTTG
 CGACGCGCGTGCAGGCGCAGACTGAGGTTGGCAACGCCAATCAGCAACGACTGTTTGGCCGCGAGTTGTTGTGCCACGCGGTTGGGAATGTAATCAGCTCCGCCATCGCGCTTCC
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 CCACCGTGAATGACTCTTTCGGCGGCTATCATGACATACCGGAAAGGTTTTGCGCCATTCGATGGTGTCCGCGATCTCGAGCTCTCCCTTATGCGACTCCTGCATTAGGAAG
 AGCCAGTAGTAGTTGAGGCGCTTGTGACCCGCGCGCAAGGAATGGTGCATGAAGGAGATGGCGCCCAACAGTCCCGCGCCAGGGGCTGCCACCATACCCACGCGGAAACA
 AGCGCTCATGAGCCGAAGTGGCGAGCCGATCTTCCCATCGGTTGATGTCGCGGATATAGCGCCAGCAACCGCACCTGTGGCGCGGATGCGCGCCAGATGCGTCCGGCGTAG
 AGGATCGAGATCTCGATCCCGGAAATTAATACGACTCACTAAGGGGAATGTGAGCGGATAACAATCCCTCTAGAAAAATTTTGTTTAACTTTAAGAAGGAGATATACCATGG
 GAGATGACGGCGCAATTCAGAGATTAAGCAAAATGGGCAATTAAGAGCCAGGATATTCAGCCCGGCTCTGAGTGGCAtgAAGCGGATGTTAATGATCAGCCGCTGAGCGGT
 CACCGATgATGGTAAACATATCATTCAGGGGCAATGATGACGTTAGTGGCACGGCTCCGGTCAATGTCACCAATAAGATGCTGTTAAAGCAGTTGAAATGCGCTGAAAAAGAGATG
 ATCGTTTATAAAGCGCGCAGGAAAAACAGTCAATCACCCTGTTTACTGATATTACCCTGGTTACGCCACAAACTGCATGAGCAAAATGGCAGACTACAACGCGCTGGGGATCACCG
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 GGCTGAGG/-/CCTCAGCTTCCGCTGAGGTCGACAACTTGGCGCGCACTCGAGCACCAACCCACCTAGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAG
 TTGGTGTGCCACCGCTGAGCAATAACTAGCATAAACCCCTTGGGGCTCTAAACGGGCTTTCAGGGGTTTTTTCGCTGAAAGGAGAACTATATCCGGAT

pHP3 sequence landmarks:

- **T7 promoter:** in gray
- **First ATG (methionine):** in yellow
- **Gene encoding an inactive DsbC:** in green
- **His-Tag coding sequences:** in purple
- **Cloning region:** ~~✂~~
- **T7 terminator:** in dark gray
- **Sequencing primers (T7 universal and T7 terminator):** underlined
- **BglII, NcoI & XhoI recognition sites:** in bold

Sequence added to the final recombinant protein (25.91 KDa):

MGDDAAIQQTLAKMGIKSSDIQAPVPMKIVLNSGVLYITDDGKHI IQGPMYDVSPTAPVNVNKMMLLQNLNALEKEMIVYKAPQEKHVI TVFTDI TAGYA
 HKLHEQMADYNALGITVRYLAFPRQGLSDAEKEMKAIWCAKDKNKAFDDVMAGKSVAPASCDVD IADHYALGVQLGVSGTPAVVLSNGTLVPGYPPKEMKE
 FLDEHQKMTSGKSSSMGSSHHHHHHSSSGPQQGLR