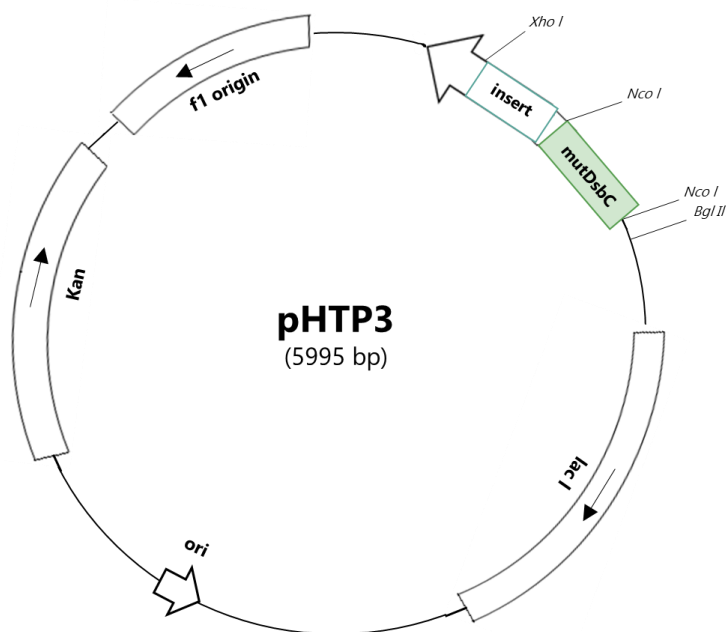


pHTP3 Expression Vector

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
CCATGGG GAGATGACGCGGCAATTCAACAAACG . 648bp . AAAATGACCAGCGGTAAAGGATCAT CCATGGG GCAGCAGCC CATCATCATCATCAC AGCAGCGGC			
MetGlyAspAspAlaAlaIleGlnGlnThr . 216aa . LysMetThrSerGlyLysGlySerSerMetGlySerSer			HisHisHisHisHisHisSerSerGly
CCTCAGCAAGGGCTGAGG /> / CCTCAGCTTCCGCTGAGGTCCGTCGACAAGCTTGCGGCCGCA		<i>Xho I</i>	His-Tag
ProGlnGlnGlyLeuArg /> / ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu			HisHisHisHisHisHis* STOP

> 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)

TGGCGAATGGGACGCGCCCTGTAGCGGGCGCATTAAAGCGCGGGGTGTGGTGGTTACGCGCAGCGTGACCCTGACACTTGCCAGCGCCCTAGCGCCCGCTCCCTTCGCTTTCT
 TCCCTTCCTTTCTCGCCACGTTTCGGGGCTTTCCCGCTCAAGCTCAAATCGGGGGCTCCCTTAGGGTTCGGATTTAGTGTCTTACGGCACCTCGACCCAAAAAACTTGAT
 TAGGGTATGGTTCACGTAGTGGGCGCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTTTGTTCAAAACCTGGAAACAACACT
 CAACCCTATCTCGGTCTATTCTTTGATTTATAAGGGATTTTCCGGATTTTCGGCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACCGGAATTTTAAACAAAATAT
 TAAGTTTACAAATTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTGTTTATTTTCTAAATACATTCAAATATGATATCCGCTCATGAATTAATCTTAGAA
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 ATAGGATGGCAAGATCCTGGTATCGGTTCGGGATTCGGACTCGTCCAACATCAATACAACCTTAATATTTCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGA
 GTGACGACTGAATCCGGTGAAGTGGCAAAAGTTTATGCATTTCTTCCAGACTTTGTTCAACAGGCCAGCCATTACGCTCGTCAATAAATCACTCGCATCAACAAAACCGTT
 ATTCATTGCTGATTCGGCTGAGCGAGACGAAATACGGGATCGCTGTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCGAGCGCATCAACAA
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 GGAAGAGGCATAAAATTCGTCAGCCAGTTAGTCTGACCATCTCATCTGTAACATCAATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTGCGGCATCGGGCTTCCC
 ATACAATCGATATTCGTCGACATTTGCCCCGACATTTATCCCGGATTTGATACCATATAAATCGACATAGCATGTTGGAATTTAATCCGCGCCCTAGAGCAAGACGTTTT
 CCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTATGACAAAATCCCTTAACGTGAGTTTTGCTTCCACTGAGCGTCAGA
 CCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAACTGCTGCTGCAAAACAAAAAACCCCGCTACACAGCGGTGGTTTTGTTGCCGATCAAG
 AGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCCAGATACCAAACTATGTCCTTCTAGTGTAGCCGTAAGTTAGGCCACCACCTCAAGAACTCTGTAGCACC
 CCTACATACCTCGCTCTGTAATCTGTACCAGTGGCTGCTGCCAGTGGCGATAAGTCTGCTCTTACCAGGTTGGACTCAAGACGATAAGTTACCGGATAAGCGCAAGCCGTC
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 CTTGAGCGTCGATTTTGTGATGCTCGTCAGGGGGGGGAGCCTATGAAAAACGCCAGCAACCGCGCCCTTTTACGGTTCCTGGCCCTTTGCTGGCCCTTTGCTCACATGTT
 CTTTCCCTGATATCCCTGATCTGAGTTTACCGTATACCGTATTACCGCCCTTTGATGATACCATATAAATCGACGATAGCATGTTGGAATTTAATCCGCGCCCTAGAGCAAGACGTTTT
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 GCACCCGTTGGGGCCCGCATGCCGCGATAATGGCTGCTTTCGCGCAACGTTTGGTGGCGGGACAGTGCAGGAGGCTTGAGCGAGGGCGTGCAAGATTCCGAATACCGCA
 AGCCACAGCCGATCATCGTCCGCTCCAGCGAAAGCGGCTCCGCGAAATGACCCAGAGCGCTGCCGCGACCTGTCTTACGAGTTCGATGATAAAGAGACAGTCATAAG
 TGGCGGACGATAGTCAATGCCCGCGCCACCGGAAGGAGCTGACTGGGTTGAAGGCTCTCAAGGCAATCGGTCGAGATCCCGGTGCCTAATGAGTGAAGTAACTTACATTA
 TTGCTTGGCTCAGCTCCGCTTTCAGTTCGCGGAAACCTGTCGTCAGCTGATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGGCTATTTGGCGCCAGGTTGGT
 TTTTCTTTTACCAGTGAAGCGGGCAACAGCTGATTGCCCTTACCGCTTGGCCCTGAGAGAGTTGCAGCAAGCGGTCACCGCTGGTTTGGCCAGCAGCGAAAAATCCTGTT
 TGATGGTGGTTAACGGCGGGATATAACATGAGCTGCTTCGGTATCGTGTATCCCACTACCGAGATATCCGACCAACGCGCAGCCCGGACTCGGTAATGGCGCGCATTGGC
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 TCATGCCATACCGGAAAGGTTTTCGCCATTTCGATGGTGTCCGGGATTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCAGTAGTAGGTTGAGGCGGTTG
 AGCACCGCGCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCGCGCCAGGGGCTGCCACCATAACCGCGGAAACAAGCCGCTCATGAGCCGAAAGT
 GCGAGCCGATCTTCCCATCGGTGATGTCGGGATATAGCGCCAGCAACCGCAGCTGCGCGCGGTTGATGCGCGCCAGCATGCGTCCGGCTAGAGGATCGAGATCTCGA
 TCCCGGAAAAATTAATACGACTCATATAGGGGAAATTTGAGCGGATAAACAATTTCCCTCTAGAAAATAAATTTGTTTAACTTTAAGAAGGAGATATA**CCATGGGAGATGACGCG**
 GCAATTCACAAACGTTAGCCAAAATGGGCATCAAAGCAGCGATATTCAGCCCGCCCTgTAGCTGGCAtgAAGACAGTTCTGACTAACAGCGCGtgTGTGATACATACCGA
 tgATGGTAAACATATCAATTCAGGGGCCAATGTATGACGTTAGTGGCAGCGCTCCGGTCAATGTCAACAATAAGATGCTGTTAAAGCAGTTGAATGCGCTGAAAAAGAGATGA
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 AGGTAAGACGCTCGCACAGCCAGTTGCGACGTGGATATTGCCGACATTACGCACTTGGCGTCCAGCTTGGCGTTAGCGGTTACTCCGGCAGTTGTGCTGAGCAATGGCACAC
 TTGTTCCGGGTTACAGCGCGCGAAAGAGATGAAAGAAATTTCTCGACGAAACAAAAAaTGACACAGCGGTAAGGATCAT**CCATGGGAGCAGCCATCATCATCATCAC**
 AGCAGCGCCCTCAGCAAGGGCTGAGG/↗/CCTCAGCTTCCGCTGAGGTCGCGACAAGCTTGGCGCCGCA**CTCGAGCACCAACCACCACCAC**TGAGATCCGGCTGCT
 AACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAA**TAGCATAACCCCTTGGGGCCCTCTAAACCGGCTTTGAGGGGTTTTTGTGCTGAAAGGAGGAAC**
 TATATCCGGAT

pHTP3 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):

MGDDAAIQQTLAKMGIKSSDIQPAPVAGMKTVLTNSGVLYITDDGKHI IQGPMYDVSGTAPVNVTKMLLKQLNALEKEMIVYKAPQEKHVITVFTDI
TAGYAHKLHEQMADYNALGITVRYLAFPRQGLSDAEKEMKAIWCAKDKNKAFDDVMAGKSVAPASCDVDIADHYALGVQLGVSGT PAVVLSNGTLVP
GYQPPKEMKEFLDEHQKMTSGKGSSMGSSHHHHHSSGFPQQGLR