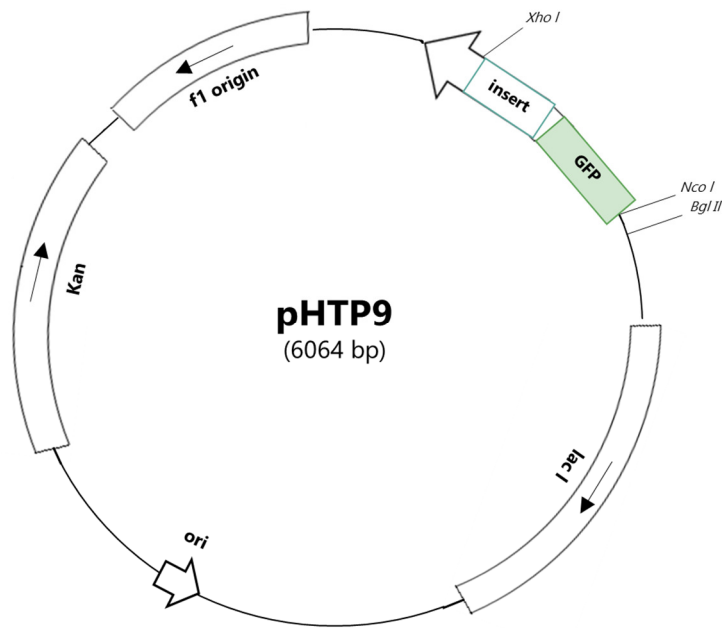




pHTP9 Vector


pHTP9 was designed for the cloning and expression of high-levels of recombinant proteins in *Escherichia coli*. Recombinant proteins are expressed in fusion with the Green Fluorescent Protein (GFP). This vector, included in the portfolio of NZYTech pHTP expression vectors, is part of the NZYEasy Cloning & Expression System. pHTP9 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



pHTP9 Cloning/Expression Region

<i>Nco I</i>	GFP	His-Tag
<u>CCATGGGAGTTAGCAAAGGTGAAGAAGCTGTTT</u> . 714bp.	<u>GATGAACTGTACAAAAGCTCTGGTCCATCGGGCAGCAGCCATCATCATCATCACAGCAGCGGC</u>	
MetGlyValSerLysGlyGluGluLeuPhe. 238aa.	AspGluLeuTyrLysSerSerGlyProSerGlySerSerHisHisHisHisHisHisSerSerGly	
CCTCAGCAAGGGCTGAGG /  /	CCTCAGCTTCCGCTGAGGTCGCGTCAAGCTTGCGGCCGCA	<u>CTCGAGCACCACCACCACCACCAC</u> TGAGATCCGGCTGCT
ProGlnGlnGlyLeuArg /  /	ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu	HisHisHisHisHisHis*

 Represents the site where the gene will be inserted.

Note: For correct expression, inserted gene needs to be in frame with pHTP9 5' gene sequence. Inserts correctly cloned into pHTP9 will maintain reading frames starting on the ATG codon.

2. Vector Sequence (6064 bp)

TGGCGAATGGGACGCGCCCTGTAGCGCGCATTAAGCGCGGGGGTGGTGGTTACGGCGACGTGACCGCTACACTTGCAGCGCCCTAGCGGCCGCTCCTTTCCGCTTCTTCCCT
 TCCTTCTCGCCAGCTTCGCGCGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTATAGGTTTCCGATTAGTGCTTTACGGCACCTCGACCCAAAAAACTTGATTAGGGTGATG
 GTTCCAGTAGTGGGACATCGCCCTGATAGACGGTTTTTTCCGCTTTGACGTTGGAGTCCACGTCTTTAATAGTGGACTCTTGTTCAGAACTGGAAACAACACTCAACCTATCTCGGT
 CTATCTTTTGATTTATAAGGGATTTTCCGCTATTGGTAAAAATGAGCTGATTTAACAAAAATTTAACCGGAATTTAAACAAAAATTTAAGCTTTTAACTTTACGCTTTTTCAGGT
 GGCACCTTTTCGGGGAATGTGCGCGGAACCCCTATTTGTTTTATTTCTAAATATCAATATGATCCGCTCAGTAATTTCTAGAAAAACTCATCGAGCATCAAAATGAAAC
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 CGATTCCGACTCTCCAACTAATCAACCTATTAATTTCCCGCTTCAAAAAATAAGTTTACAGTGAAGAAATCACCATGAGTACAGTGAATCCGGTGAGAAATGGCAAAAGTTT
 ATGCATTTCTTCCAGACTTGTTCACAGCCGACCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAACCGTTATTCATTCTGTTATGCGCCTGAGCGAGACGAAATACGCGA
 TCCTGTAAAAAGGACAAATACAAACAGGAATCGAATGCAACCCGGCGCAGGAACTGCCAGGCATCAACAATATTTTACCTGAATCAGGATATTTCTTAAATACCTGGAATGCTG
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 AACATCATTTGGCAACGCTACCTTTGCCATGTTCCAGAAAACACTCTGCGCATCGGGCTCCCATACAATCGATAGATTGTCGACCTGATTGCCCGCAATTATCGCGAGCCATTTA
 TACCCATATAAATCAGCATCCATGTTGAATTTAATCGCGCCCTAGAGCAAGACGTTCCCGTTGAATATGGCTCATAACACCCCTGTTATTACTGTTATGTAAGCAGACAGTTTA
 TTGTCATGACAAAAATCCCTTAACGTGAGTTTTCTGTCCACTGAGCGTCAGACCCCGTAGAAAAAGTCAAAGGATCTTCTTGTGATCCTTTTTCTGCGCGTAACTGTGCTTGC
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 AGTCAGTGAAGAGGAAAGCGGACGCTGATGCGGTTATTTCTCCTTACGATCTGTGCGGTTTCCACCGCATATAAGTGCCTTCTCAGTACAATCTGCTGATGCCGCA
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 TTAACGTTCTGCTCGGCTATCGGTGATTAATCTGCTAACCAGTAAAGCAACCCCGCAGCTGAGCGGCTTCCAAAGCAGAGGACGATCATCGCCACCCGTTGGGGCCCGCATGCC
 GGCATAATGGCCTGCTTCTGCGGAAACGTTTGGTGGCGGACAGTACGAAGGCTTGGAGCGGCGGCTGCAAGATTCCGAATACCAGAACGACAGGCGGATCATCGTCGCGCTC
 CAGCGAAAGCGGCTCTCCGCGAAAAATGACCCAGAGCCTGCGGCGCACTGTCTACGAGTTGCAATGATAAAGAGAGCAGTATAAGTGGCGGACGATAGTATGCCCGCGCCCAA
 GGAAGGAGTACTGGTTGAAGCTCTCAAGGCATCGGTCGAGATCCCGGTGCCTAATGAGTGAAGTAATTAATTTGCGTTCAGTCCCTGCTTCCAGTCCGGGAA
 CCTGTGTCGACAGCAGTAATAATGAGCCCGCTTAACAGCGCATTTGCTGTTGACCAATGCGCAGATGTCTCCAGCCAGTCCGCTTTCATGGGAGAAAAAATACTGTTG
 CACCCTGCGCCCTGAGAGAGTTGACAGAACGGTCCACGCTGTTTGGCCAGCAGGCAAAATCTGTTTATGTTGGTTAAACCGCGGGATATAACATGAGTGTCTTCCGTATCG
 TCGTATCCCACTACCGAGATATCCGCACCAACGCGCAGCCGACTCGGTAATGGCGCATAGTGCAGCGCCTGTTGAGTGGGTTAAGCAGCGGATAGTGGGAAACAGTGCCT
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 AGCGCTCATGAGCCGAAGTGGCGAGCCGATTTCCCATCGGTGATGTCCGCGATATAGCGCCAGCAACCCGACCTGTGGCGCGGTTGATCCGGCCAGTGCCTGCGGCGTAG
 AGGATCCAGATCTCGATCCCGGAAATTAATAGACTCACTATAGGGGAATGTGAGCGGATAACAATCCCTCTAGAAAAATTTTGTTTAATTTAAGAAAGGAGATATACCATGG
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 GACAATCATTATCTGCCACCCAGTCAAGCTGTGCAAGATCCGAATGAAAACCGGACCACATGGTGTCTGGAATTTGTTACCAGCGCCGTTATACGCTGGCGATGGATGAAC
 TGTACAAAAGCTCTGTTCCATCGGCAAGCAGCATCATTATCATTACAGCAGCGCCCTCAGCAAGGGCTGAGG/❄/CCTCAGCTTCCGCTGAGTCCGCTGACAAGCTTGC
 GCGCACTCGAGCACCACCACCCACCTGAGATCCGCTGCTAACAAGCCCGAAAGGAAGCTGAGTTGGCTGTGCCACCGCTGAGCAATAAACTAGCATAAACCCCTTGGGGCT
 CTAAACGGGCTTTGAGGGGTTTTTTCGTGAAAGGAGGAACATATATCCGGAT

pHP9 sequence landmarks:

- **T7 promoter:** in gray
- **First ATG (methionine):** in yellow
- **Green Fluorescent Protein (GFP) gene:** in green
- **His*Tag coding sequences:** in purple
- **Cloning region:** ❄
- **T7 terminator:** in dark gray
- **Sequencing primers (T7 universal and T7 terminator):** underlined
- **BglIII, NcoI & XhoI recognition sites:** in bold

Sequence added to the final recombinant protein (29.33 KDa):

MGVSKGEELFTGVVPIIIVELDGDVNGHKFVSSEGEEDATYKGLTLKFICTGKLPVWPVLVTTTLTYGVQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFK
 DDGNYKTRAEVKEFDGLTVNRILKIDFKEDGNILGKLEYNYNVSHKVIITADKQKNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQ
 SALSALPNEKRDMVLELVTAAGITLGMDELYKSSGPSGSHHHHHSSGPPQQLR