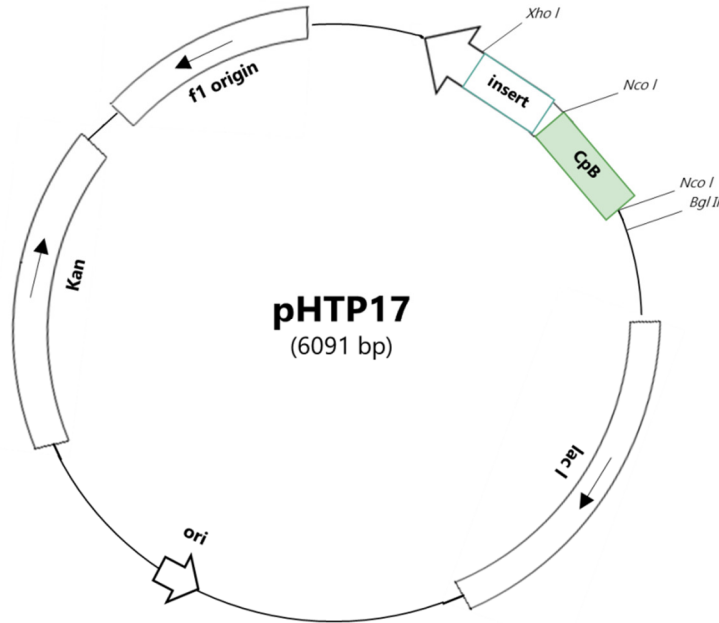


pHTP17 Expression Vector

pHTP17 was designed for the cloning and expression of high-levels of recombinant proteins in *Escherichia coli*. Recombinant proteins are expressed in fusion with a *Ruminococcus flavefaciens* cellulosomal protein (CpB), which is able to *promote solubility* and folding of fusion partners. This vector, included in the portfolio of NZYTech pHTP expression vectors, is part of the NZYEasy Cloning & Expression System. pHTP17 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



pHTP17 Cloning/Expression Region

Nco I	CpB	Nco I	His-Tag
<u>CCATGGG</u> TGTCAAAAAGGAAACTCCTAACCTC	.750bp..TCAAAGATATTCAATACAGCAACTG	<u>CCATGGG</u> CAGCAGCCATCATCATCATCACAGCAGCGGC	
MetGlyValLysLysGluThrProAsnLeu	.250aa..SerLysIlePheAsnThrAlaThrAla	MetGlySerSer	HisHisHisHisHisHisSerSerGly
CCTCAGCAAGGGCTGAGG	/Xho I/ CCTCAGCTTCCGCTGAGGTCCGTCGACAAGCTTGC	CGCCAC	HisHisHisHisHisHis
ProGlnGlnGlyLeuArg	/Xho I/ ProGlnLeuProLeuArgSerValAspLysLeuAlaAlaAlaLeuGlu		HisHisHisHisHisHis*

Xho I Represents the site where the gene will be inserted.


Note 1: For correct expression, inserted gene needs to be in frame with pHTP17 5' gene sequence. Inserts correctly cloned into pHTP17 will maintain reading frames starting on the ATG codon.

Note 2: CpB is a recombinant cellulosomal protein (Cp) that is highly expressed in *E. coli*.

2. Vector Sequence (6091 bp)

TGGCGAATGGGACGGCCCTGTAGCGGCGCATTAAAGCGGGGGGTGGTGGTTACGCGCAGCGTGACCCTACACTTGCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTCCCT
 TCCTTTCTCGCCACGTTTCGCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATG
 GTTCACGTAAGTGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTTGTCCAACTGGAACAACACTCAACCTATCTCGGT
 CTATTCCTTTGATTATAAGGGATTTGCGGATTTGCGCCATTGGTTAAAAAATGAGCTGATTAAACAAAAATTAACCGGAATTTAAACAAAAATATAACGTTTACAATTCAGGT
 GGCACCTTTTCGGGAAATGTGCGCGAACCCCTATTTGTTATTTTCTAAATACATCAAAATATGTATCCGCTCATGAATTAATCTTAGAAAACTCATCGAGCATCAAAATGAAAC
 TGCAAATTTATCATATCAGGATTATCAATACCATATTTTGGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTACCAGGCGAGTCCATAGGATGGCAAGATCCTGGTATCGGCTG
 CGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGTCAAAAAAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTT
 ATGCATTTCTTCCAGACTTGTTCACAGGCCAGCCATTACGCTCGTCAAAATCACTCGCATCAACCAACCCGTTATTCATTCTGATGTCGCTGAGCGAGACGAAATACCGGA
 TCGCTGTTAAAAAGGACAATTACAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTAATACCTGGAATGCTG
 TTTTCCCGGGGATCGCAGTGGTGAGTAACCATGTCATCATCAGGATGTCGATAAAATGCTTGGTGGTGGGAGAGGCATAAAATCCGTCAGCGAGTTTGTCTGACCATCTCATCTGT
 AACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAAACTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTA
 TACCCATATAAATCAGCATCCATGTTGGAAATTAATCGCGCCCTAGAGCAAGAGCTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTAA
 TTGTTTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAAGATCAAAAGGATCTTCTTGGATCCTTTTTTTCGCGGTAATCTGTGCTTGC
 AACAAAAAACCACCCCTACCAGGCTGTTGTTGTCGGGATCAGAGATCAACACTCTTTTCCGAAAGTCACTGGCTTCAGCAGAGCAGATACCAAACTGCTCTCTAGT
 GTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGTCTGCTAATCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAAGTCTGTCTTACCGGTGTG
 GACTCAAGACGATAGTTACCAGGATAAGCGCGAGCGGTGCGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAAGTGAATACCTACAGCGTGAGC
 TATGAGAAAGCGCCACGCTTCCGGAAGGAGAAAGCGGACAGGATCCGCTAAGCGCGAGGTTCCGAAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCTTGGTATCTTTA
 TAGTCTGTCCGGTTTCGCACTTACTGACTGAGCGTCGATTTTGTGATGCTCGTCAAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGCCCTTTTACGGTTCTGTGCGCTTT
 TGCTGCGCTTTGCTCATTGCTTCTTCCGCTGATTTCTGTTGTAACCGTATTAACCGTATTAACCGTATTAACCGTATTAACCGTATTAACCGTATTAACCGTATTAACCGTATTAACCGT
 AGTCAGTGAAGCGGAAAGCGGACGCGCTGATCGGTTATTTCTCTTACGCATCTGTGCGGTTATTCACACCGCATATATGGTGCACCTCAGTACAATCTGCTCTGATCGCCGA
 TAGTTAAGCCAGTATACACTCCGCTATCGCTACGTGAGTGGGTATGGCTGCGCCGACACCCGCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGCATCCGCTTA
 CAGACAAGCTGTGACCTCTCCGGGATGTCATGTCAGAGGTTTCCACCTCATACCGAAACCGCGAGGCGAGCTGCGGTAAGCTCATCAGCTGTGCTGTAAGCGATTACAG
 ATGTCGCTGCTGCTCATCCGCTCAGCTCAGCTTCTGAGTTTCTCCAGAAAGCTTAATGTCTGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTCTGTTTGGTCACTGATGCTT
 CCGTGAAGGGGATTTCTGTTTCATGGGGTAAATGATACCGATGAAACGAGAGAGGATGCTCACGATACGGGTTACTGATGATGAACATGCCCGGTTACTGGAACGTTGTGAGGGTAA
 ACAACTGGCGGATGATGATGCGGGGGACAGAGAAAAATCACTCAGGTCATGCCAGCGCTCGTTAATACAGATGTAGGTGTTCCACAGGGTAGCCAGCAGCATCTCGCATGACG
 ATCCGGAACATAATGGTGCAGGGCGCTGACTTCCCGCTTCCAGACTTTACGAAACCGGAAACCGAAGACCATTCATGTTGTTGCTCAGTTCGAGACGTTTTCGAGCAGCAGTCCG
 TTCAGTTCGCTCAGCACTTAATGGGCGCCGTAACAGCGGATTTCTGCTAACCAAGTAAAGCAACCGCCAGCCGCTTCCGCGGCTTCTGATAAAGCGGGCCATGTTAAGGGCGGTTTTTCTGTTTGGTCACTGATGCTT
 GCGGATAATGGCTGCTTCTCGCGAAACGTTTGGTGGCGGACAGTACGAAAGCTTGGAGCGGGGCTGCAAGATTCCGAAATACCGCAAGCGACAGGCGCATCATCTGCTCGGCTC
 CAGCGAAAGCGGCTCCTCGCGAAATGACCCAGAGCGCTGCGCGCACTGCTTACGAGTTGATGATAAAGAGACAGTATAAGTGGCGGACGATAGTATGCCCGCGCCACC
 GGAAGGAGCTGACTGGTTGAAGGCTCTCAAGGCGATCGGTCGAGATCCCGGTGCTAATGAGTGAAGTAACTTACATTAATGCGTTGCGCTCACTGCCGCTTCCAGTCCGGAA
 ACTGTCGTCGCTGCTCAATTAAGTAACTGGCCAAACGCGCGGGGAGAGCGGTTTGGCTATTGGCGCCGAGGGTGGTTTTCTTTTCCACCGTGAAGACGGGCAACAGCTGATGCTGCTT
 CACCGCTGGCCCTGAGAGAGTTGACGAAAGCGGTCACGCTGGTTTGGCCAGCAGCGGAAATCCTGTTTGTGTTGGTAAACGGCGGGATATAACATGAGCTGTCTCGGTATCG
 TCGTATCCCACTACCGAGATATCCGCAACAGCGCAGCCGCGATCGGTAATGGCGCGCATTGGCGCCAGCGCCATCTGATCGTTGGCAACAGCATCGCATGGGAACGATGCCCT
 CATTACGATTTGATGTTTGTGAAAAACCGGACATGGCACTCCGCTCCCGTTCGCTATCGGCTGAATTTGATTGCGAGTGAATATTTATGCCAGCCAGCCAGAGCGCAG
 ACCGCGCAGACAGAACTTAATGGGCGCCGTAACAGCGGATTTGCTGTTGACCTAAGCGGCAACCGGATGCTGTCACAGCAGATGCTCCACGCGCCAGTCCGCTACCGCTTTCATGTTG
 ATGGGTGCTGGTCAGAGACATCAAGAAATAACCGGAAACATAGTGCAGGCGAGTCCACAGCAATGGCATCCTGGTCACTCCAGCGGATAGTTAATGATCAGCCACTGACCGGTT
 GCGCGAGAAGATTGTGACCGCGCTTTACAGGCTTCGACGCGCTTCTGTTTACCATCGACACCACACGCTGGCACCAGTTGATCGGCGGAGATTAAATCGCCCGGACAAATTTG
 CGACGGCGGTCGAGGGCCAGACTGGAGGTGGCAACGCCAATCAGCAACGACTGTTTGGCCCGCAGTTGTTGTGCCACGCGGTTGGGAATGTAATTCAGCTCCGCCATCGCCGCTTCA
 ACTTTTCCCGGTTTTTCGAGAAACGTTGCGCTGGTTTCACACCGCGGAAACCGTCTGATGAAAGACAGCCAGCCGATACCTGCGACATCGTATAAAGTACTGTTTACATTCACATTC
 CCACCTGAAATGACTCTTCCGGGCGCTATCATGCCATACCGGAAAGGTTTTGCGCCATTGATGGTTCGCGGATCTCGACGCTCTCCCTTATGCGACTCTGCAATAGGAAGC
 AGCCAGTAGTAGTTGAGGCGGTTGAGCACCGCGCCGCAAGGAAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCGCGCCAGCGGGCTGCCACCATACCCAGCCGAAAC
 AGCGCTCATGAGCCGAAGTGGCGAGCCGATCTTCCCATCGGTGATGTCGGGATATAGGCGCCAGCAACCCGACCTGTGGCGCGGTTGATCGCCGCAACGATGCGCTCCGGCTAG
 AGGATCGAGACTCGATCCCGGAAATTAATACGACTCATAAGGGAAATGGGCGAATGAGGAGTAAACAAATCCCTTAGAAAATAATTTGTTTAAATTAAGAAAGGATATAACATG
 TGTCAAAAAGGAACTCCTAACCTTCTTCTTAAGGGAAAGGAAATGAGTATACAGGTTTCGCTTTCAGAACTTATCGAAAGCAGGAAAGGCTTTCGGGCGGTACACTTCAGTATAAG
 ATAGGCGAGAACCGCGAATGGAGTGAAGATCCCGACTGCTGCGGAAAGTGGAGACTATACAGTTTACTACAGAGTTGTCGGCAATGAAAAGTATTACGGTGCAGACACGGATGCGG
 ATATTAAGGGCAGAAAGAACTATTTCTTTTCCCTACTGCTATGACTGTGAGAGAAACCTGACATCTTCAAAGGGTGAAAGCTCGAGAGCATACAGTTAAAAGAACCTCTTATCGG
 CGATCGTGTTTTCGGCAAGAAATAATCAAAAGAACTGGACGATGGAATATGTTTCAGCTTATAAAGATTTTGAATCACCATCAAAGTATCTTATAGGCTTGTAGAAAAACTCAG
 GAGGAGATTCACTCAGTACTCCGATGATATTTCCATCTATGAATTAAGAGGAGTGGCGAGCATAAATCTTATGGTGTGTTATTGCAAGTACAGAGGGTGAAGAATGAACTCTTT
 ACTTTGGCGATACAATAAATATGGCGGAGGATATCTCTCAAAGGAAGCAATAAGTGCAGTAAAGACATTGCTCATGAAAAAGACCTGAAAGAAATCATTTGTTGAGACAGTAAA
 GCTTTCGGGTACAGTAAATCAAAAGATTTCAATACAGCAACTGCCATGGGCGACAGCCATCATCATCATCACAGCAGCGCCCTCAGCAAGGGCTGAGG/Δ/CCTCAGCTTC
 CGCTGAGGTCGTCGAAAGCTTGGCGCCGACTCGAGCACCCACCACCACCTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAAGTGAAGTGGTGTGCTGCCACCGCTGAGCA
 ATAACATAGCATAAACCCTTGGGGCCCTAAACGGGCTTGTAGGGGTTTTTGTCTGAAGAGGAACTATATCCGGAT

pHTP17 sequence landmarks:

- **T7 promoter:** in gray
- **First ATG (methionine):** in yellow
- **CpB gene:** 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 (30.24 kDa):

MGVKKETPNLILPKGKEMEYTGSLQELIEAGKASGGTLQYKIGENGEWSEKIPTAAEVGDYTVYYRVVGNKYGGADTDADIKGRKNYSFPYCYDCQRNPAHP
 SKGESCRAYQLKEPLIGDRVFGKNI IKGNWIMEYVSAYKDFESP SKYLIGLVEKTEADSLSTPDDIS IYELKRDGEHNSYGVVIAVSEGENELLYFGDTIKY
 GGGYILSKEAISDSKTFVIEKDLKEIIVETVKLSGTVNSKIFNTATAMGSSHHHHHHSSGPQQLR