

pET-28a-c(+) Vectors

	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

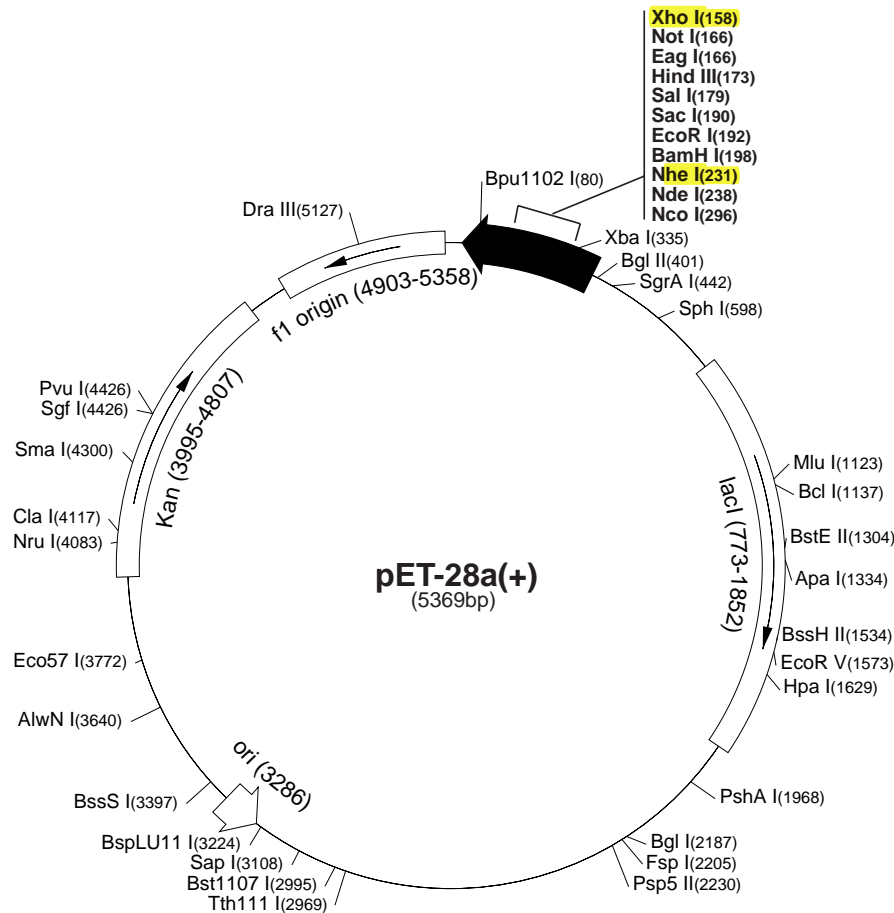
The pET-28a-c(+) vectors carry an N-terminal His•Tag[®]/thrombin/T7•Tag[®] configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

INTS3 C-termini (514-1042 aa) was cloned into NheI and XhoI site

pET-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites (<i>Bam</i> H I - <i>Xho</i> I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
<i>lac</i> I coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.



T7 promoter primer #69348-3

pET upstream primer #69214-3 → *Bgl*II →

T7 promoter → lac operator → *Xba*I → rbs

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AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCGCCCTAGAAATAATTTGTTAACTTTAAGAAGGAGA
      Nco I          His•Tag          Nde I  Nhe I          T7•Tag
TATACCATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGC CGCGCGGCAGCCATATGGCTAGCATGACTGGTGACAGCAA
MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetAlaSerMetThrGlyGlyGlnGln
      thrombin
      His•Tag
ATGGGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28a(+)
MetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProLeuArgSerGlyCysEnd
      BamHI EcoRI SacI SalI Hind III      Eag I  Xho I
      Not I
...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28b(+)
...GlyArgAspProAsnSerSerSerValAspLysLeuAlaAlaAlaLeuGluHisHisHisHisHisHisEnd
...GGTCGGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCAGCTGAGATCCGGCTGCTAACAAAGCCC pET-28c(+)
...GlyArgIleArgIleArgAlaProSerThrSerLeuArgProHisSerSerThrThrThrThrGluIleArgLeuLeuThrLysPro...
      Bpu1102 I          T7 terminator
GAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGCTTGAGGGGTTTTTTG
      T7 terminator primer #69337-3
    
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pET-28a-c(+) cloning/expression region