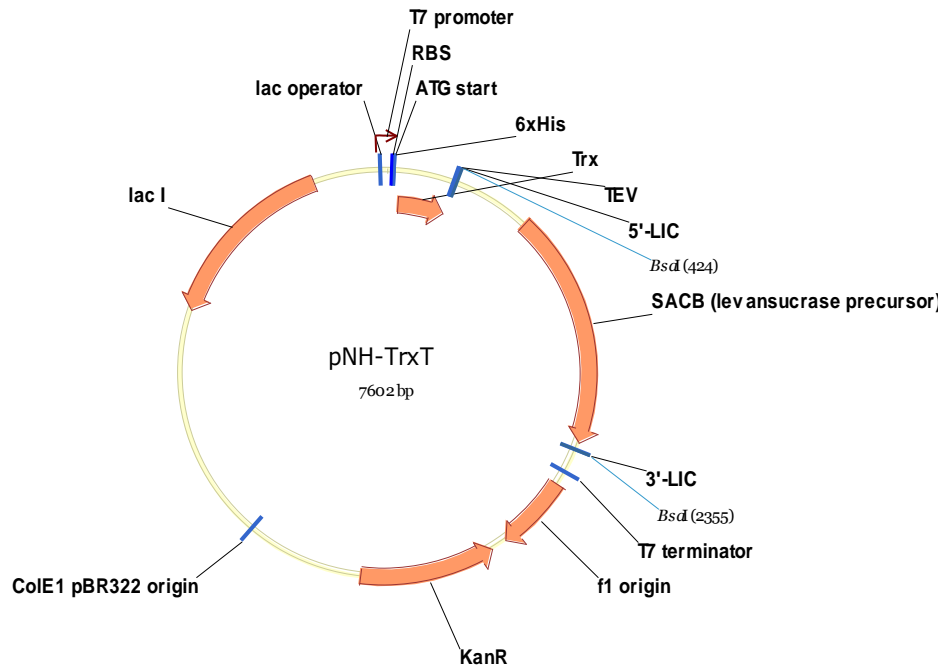


Vector information sheet

Dated: 8th May 2013

Vector Name	pNH-TrxT
Source	Chris Cooper/ Opher Gileadi
Sequence accession/link	GenBank GU269914
Description	pET expression vector with His ₆ -Trx (<i>E. coli</i> thioredoxin) in 128-aa N-terminal fusion peptide, with TEV protease cleavage site. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose
Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with BsaI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHSSGMSDKIIHLTDDSFDTDVLKADGAILVDFWAE WCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYG IRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGTEN LYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	14163.1 Da including Met (13944.8 Da removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC
Sequencing note:	5' sequencing with above primer will include all the Trx tag



Polylinker region:

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                                     pLIC-Forward
                                     ----->
                                     lac operator
                                     ~~~~~~
7543  GATCCCGCGA AATTAATACG ACTCACTATA GGGGAATTGT GAGCGGATAA CAATCCCCT
      CTAGGGCGCT TTAATTATGC TGAGTGATAT CCCCTTAAACA CTCGCCTATT GTTAAGGGGA

->
XbaI                                     NdeI
~~~~~                                     ~~~~~~
1    CTAGAAATAA TTTTGTTTAA CTTTAAAGAAG GAGATATACA TATGCACCAT CATCATCATC
     GATCTTTTAT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG

61   · S S G M S D K I I H L T D D S F D T D V
     ATTCTTCTGG TATGAGCGAT AAAATTATTC ACCTGACTGA CGACAGTTTT GACACGGATG
     TAAGAAGACC ATACTCGCTA TTTTAATAAG TGGACTGACT GCTGTCAAAA CTGTGCCTAC

121  · L K A D G A I L V D F W A E W C G P C K
     TACTCAAAGC GGACGGGGCG ATCCTCGTGC ATTTCTGGGC AGAGTGGTGC GGTCCGTGCA
     ATGAGTTTTCG CCTGCCCCGC TAGGAGCAGC TAAAGACCCG TCTACCACG CCAGGCACGT

181  · M I A P I L D E I A D E Y Q G K L T V A
     AAATGATCGC CCCGATTCTG GATGAAATCG CTGACGAATA TCAGGGCAAA CTGACCGTTG
     TTTACTAGCG GGGCTAAGAC CTACTTTAGC GACTGCTTAT AGTCCCGTTT GACTGGCAAC

241  · K L N I D Q N P G T A P K Y G I R G I P
     CAAAAGTCAA CATCGATCAA AACCTGGCA CTGCGCCGAA ATATGGCATC CGTGGTATCC
     GTTTTGACTT GTAGCTAGTT TTGGGACCGT GACGCGCTT TATACCGTAG GCACCATAGG

301  · T L L L F K N G E V A A T K V G A L S K
     CGACTCTGCT GCTGTTCAA A A C G G T G A A G T G G C G G C A A C A A A G T G G G C G C A C T G T C T A
     GCTGAGACGA CGACAAGTTT TTGCCACTTC ACCGCCGTTG GTTTCACCCG CGTGACAGAT

                                     Upper-LIC
                                     ~~~~~~

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      · G Q L K E F L D A N L A G T E N L Y F Q ·
361  AAGGTCAGTT GAAAGAGTTC CTCGACGCTA ACCTGGCCGG TACCGAGAAC TTGTA CTTC
      TTCCAGTCAA CTTTCTCAAG GAGCTGCGAT TGGACCGGCC ATGGCTCTTG AACATGAAGG

Upper-LIC
~~~~~ BsaI
· S _____

421  AATCCATGGA GACCGACGTC CACAT ..... (SacB fragment) .....
      TTAGGTACCT CTGGCTGCAG GTGTA

                                           EcoRI
                                           ~~~~~ SacI
BsaI      Lower-LIC      BamHI ~~~~~
2328  GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
      CTATAGGATA ACCGTAAC TG CAGAGGTCA TTCCACCTA TGCCTAGGCT TAAGCTCGAG

Sali
      HindIII
      *****
2388  CGTCGACAAG CTTGCGGCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
      GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG
                                           T7-reverse
                                           ←-----
2448  TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
      ATTGTTTCGG GCTTTCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT
      ←-----
                                           pLIC-rev

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Primers for LIC cloning:

Upstream: add TACTTCCAATCCATG to the 5' end (ATG in-frame with the desired coding sequence).

Downstream: add TATCCACCTTTACTG to 5' end of downstream primer; add termination codon, if necessary.

pNH-TrxT sequence:

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ctagaataatTTTTgtttaaactttaagaaggagatatacatatgcaccatcatcatcatcattcttctg
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