Vector information sheet

Dated: 8th May 2013

Vector Name	**pGTVL2**
Source	Pavel Savitsky
Sequence accession/link	

Description	pET expression vector with His$_6$ and GST tag at the N-terminal fusion, followed by 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.	MHHHHHHSSMSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGD KWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRL CHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEA IPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSSSGVDLGTENLYFQ SM (* - TEV cleavage site)
N-terminal fusion – MW	28441 Da including Met
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pGEX-for: ATAGCATGGCCTTTGCAGG
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC or T7R

T7 promoter

lac operator

*Nde*I (41)

His6-GST-TEV

*Sac*I (730)

5'-LIC

*Bsd*I (769)

*Nco*I (770)

lac I

pGTVL2

7947 bp

SACB (lev ansucrase precursor)

3'-LIC

*Bsd*I (2700)

*Bam*HI (2716)

*Eco*RI (2722)

*Sac*I (2732)

*Sal*I (2735)

*Hind*III (2741)

*Not*I (2748)

*Xho*I (2756)

T7 terminator

ColE1 pBR322 origin

f1 origin
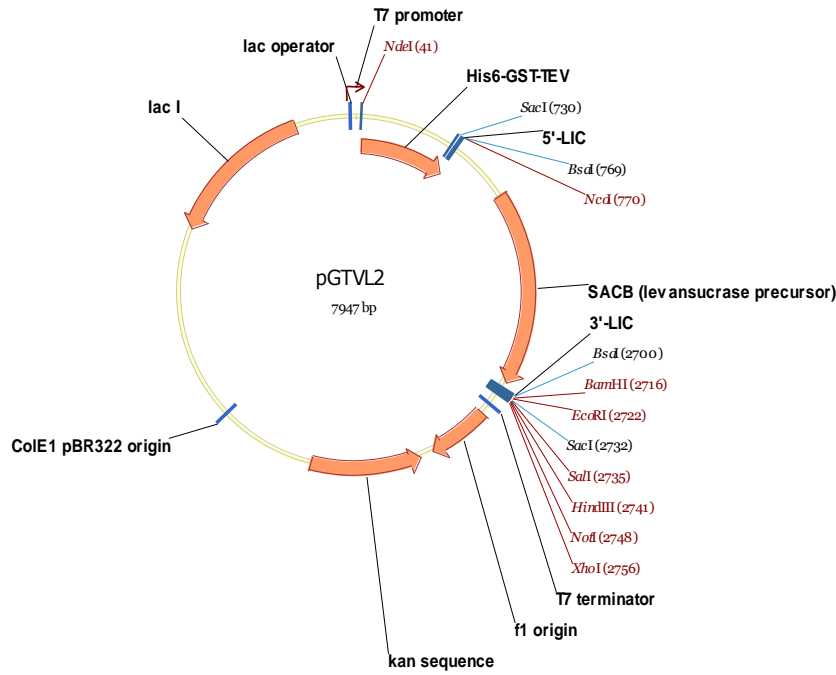
kan sequence

Tag and Polylinker sequence (SacB fragment not shown):

```
                                                   M  H  H    H  H  H  H  ·
  1  CTAGAAATAA TTTTGTTTAA CTTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC
     GATCTTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG
        S  S  M    S  P  I    L  G  Y  W    K  I  K    G  L  V    Q  P  T  R  ·
 61  ATTCTTCTAT GTCCCCTATA CTAGGTTATT GGAAAATTAA GGGCCTTGTG CAACCCACTC
     TAAGAAGATA CAGGGGATAT GATCCAATAA CCTTTTAATT CCCGGAACAC GTTGGGTGAG
        L  L  L    E  Y  L    E  E  K  Y    E  E  H    L  Y  E    R  D  E  G  ·
121  GACTTCTTTT GGAATATCTT GAAGAAAAAT ATGAAGAGCA TTTGTATGAG CGCGATGAAG
     CTGAAGAAAA CCTTATAGAA CTTCTTTTTA TACTTCTCGT AAACATACTC GCGCTACTTC
        D  K  W    R  N  K    K  F  E  L    G  L  E    F  P  N    L  P  Y  Y  ·
181  GTGATAAATG GCGAAACAAA AAGTTTGAAT GGGTTTGGA  GTTTCCCAAT CTTCCTTATT
     CACTATTTAC CGCTTTGTTT TTCAAACTTA ACCCAAACCT CAAAGGGTTA GAAGGAATAA
        I  D  G    D  V  K    L  T  Q  S    M  A  I    I  R  Y    I  A  D  K  ·
241  ATATTGATGG TGATGTTAAA TTAACACAGT CTATGGCCAT CATACGTTAT ATAGCTGACA
     TATAACTACC ACTACAATTT AATTGTGTCA GATACCGGTA GTATGCAATA TATCGACTGT
        H  N  M    L  G  G    C  P  K  E    R  A  E    I  S  M    L  E  G  A  ·
301  AGCACAACAT GTTGGGTGGT TGTCCAAAAG AGCGTGCAGA GATTTCAATG CTTGAAGGAG
     TCGTGTTGTA CAACCCACCA ACAGGTTTTC TCGCACGTCT CTAAAGTTAC GAACTTCCTC
        V  L  D    I  R  Y    G  V  S  R    I  A  Y    S  K  D    F  E  T  L  ·
361  CGGTTTTGGA TATTAGATAC GGTGTTTCGA GAATTGCATA TAGTAAAGAC TTTGAAACTC
     GCCAAAACCT ATAATCTATG CCACAAAGCT CTTAACGTAT ATCATTTCTG AAACTTTGAG
        K  V  D    F  L  S    K  L  P  E    M  L  K    M  F  E    D  R  L  C  ·
421  TCAAAGTTGA TTTTCTTAGC AAGCTACCTG AAATGCTGAA AATGTTCGAA GATCGTTTAT
     AGTTTCAACT AAAAGAATCG TTCGATGGAC TTTACGACTT TTACAAGCTT CTAGCAAATA
        H  K  T    Y  L  N    G  D  H  V    T  H  P    D  F  M    L  Y  D  A  ·
481  GTCATAAAAC ATATTTAAAT GGTGATCATG TAACCCATCC TGACTTCATG TTGTATGACG
     CAGTATTTTG TATAAATTTA CCACTAGTAC ATTGGGTAGG ACTGAAGTAC AACATACTGC
        L  D  V    V  L  Y    M  D  P  M    C  L  D    A  F  P    K  L  V  C  ·
541  CTCTTGATGT TGTTTTATAC ATGGACCCAA TGTGCCTGGA TGCGTTCCCA AAATTAGTTT
     GAGAACTACA ACAAAAATATG TACCTGGGTT ACACGGACCT ACGCAAGGGT TTTAATCAAA
        F  K  K    R  I  E    A  I  P  Q    I  D  K    Y  L  K    S  S  K  Y  ·
601  GTTTTAAAAA ACGTATTGAA GCTATCCCAC AAATTGATAA GTACTTGAAA TCCAGCAAGT
     CAAAATTTTT TGCATAACTT CGATAGGGTG TTTAACTATT CATGAACTTT AGGTCGTTCA

        pGEX-for
     ------------------->
        I  A  W    P  L  Q    G  W  Q  A    T  F  G    G  G  D    H  P  P  K  ·
661  ATATAGCATG GCCTTTGCAG GGCTGGCAAG CCACGTTTGG TGGTGGCGAC CATCCTCCAA
     TATATCGTAC CGGAAACGTC CCGACCGTTC GGTGCAAACC ACCACCGCTG GTAGGAGGTT
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                                                Lic5              BsaI
                                             ~~~~~~~~~~~~~~
          S   S   S   G   V   D   L   G   T   E   N   L   Y   F   Q   S   M   _____
    721 AATCGAGCTC AGGTGTAGAT CTGGGTACCG AGAACCTGTA CTTCCAATCC ATGGAGACCG
        TTAGCTCGAG TCCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC


    118   ----------------  (SacB fragment)  ----------------
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                                                        EcoRI
                       BsaI     LIC 3'        BamHI ~~~~~~ SacI
                       _____  ~~~~~~~~~~~~~~~ ~~~~~~      ~~~~~~
   2010 GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTCGAGCTC
        CTATAGGATA ACCGTAACTG CCAGAGGTCA TTTCCACCTA TGCCTAGGCT TAAGCTCGAG

          SalI
             HindIII
          ******~~~~~~~
   2070 CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCAC CACCACTGAG ATCCGGCTGC
        GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG
                                                      T7-reverse
                                               ←----------------
   2130 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA
        ATTGTTTCGG GCTTTCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT
               ←------------------
                    pLIC-rev
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Primers for LIC cloning:

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

Downstream: add TATCCACCTTTACTGtca to 5' end of downstream primer; (the final tca provides a termination codon)

pGTVL2 sequence:

```
ctagaaataattttgtttaactttaagaaggagatatacatatgcaccatcatcatcatcattcttcta
tgtcccctatactaggttattggaaaattaagggccttgtgcaacccactcgacttcttttggaatatc
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