

Vector pETDuet-1-LIC1

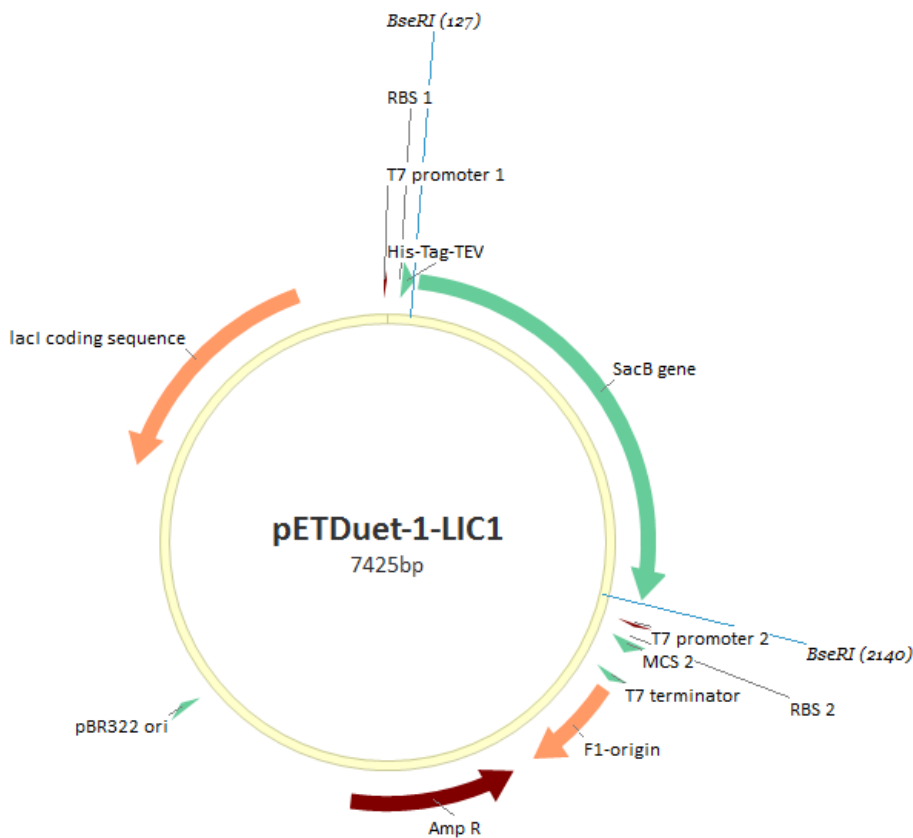
Updated in September 2015

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	pETDuet-1-LIC1 is derived from pETDuet-1 (Novagen #71146-3) designed for the coexpression of two target genes. The vector has two transcription units, the first one is modified as N-terminal fusion His-tag followed by a TEV cleavage site and the second one remains the same as original.
Antibiotic resistance	Amp R
Promoter	<i>T7lac</i> promoter
Cloning Methods	Insertion of DNA sequence into the 1 st cloning/expression region is performed using BD-Biosciences Infusion enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	ATG site in vector at bp 71
N – terminal fusion sequence	MHHHHHSSGRENLYFQG
Termination codons	TGATGA included in 3' PCR primer addition
5' primer addition for amplification of insert 1	5' TTGTATTTCCAGGGC 3'
3' primer addition for amplification of insert 1	5' CAAGCTTCGTCATCA 3
5' sequencing primer for pETDuet-1-LIC1	5' ATGCGTCCGGCGTAG 3'
3' sequencing primer for pETDuet-1-LIC1	5' GATTATGCGGCCGTGTACAA 3'

pETDuet-1-LIC1 feature Locations:

- 1st RBS: 58-63 bp
- His-Tag-TEV: 71-124 bp
- SacB gene: 141-2123 bp
- T7 promoter 2: 2219-2235 bp
- 2nd RBS: 2291-2296 bp
- MCS2: 2302-2437 bp
- T7 terminator: 2467-2514 bp
- F1 origin: 2552-2999 bp
- Amp R (complementary): 3121-3981 bp
- lacI coding sequence(complementary): 5936-7018 bp
- T7 promoter 1: 7409-7425 bp

Map of pETDuet-1-LIC1



pETDuet-1-LIC1 cloning/expression region

T7 promoter-1 lac operator

7394 tcgatccgc gaaattaata cgactcacta taaggggaat tgtgagcggg
agctagggcg ctttaattat gctgagtgat attcccctta acactcgct

rbs

18 taacaattcc cctctagaaa taattttggt taactttaag aaggagatat
attgtaagg ggagatcttt attaaaacaa attgaaattc ttcctctata

M H H H H H H S S G R E N L Y F

68 accatgcatc atcatcatca tcacagcagc ggcagagaaa acttgatatt
tggtagtagt tagtagtagt agtgctcgtc cgtctcttt tgaacataaa

Q G NdeI BseRI

118 ccagggc/cat atgagtt **ctcctc**-----SACB cassette(2 kb)---
Ggtcccg/gta tactcaa **gaggag**

BseRI 2x stop HindIII

2124 **gaggag**atca tgcaca/**tgat gacgaagctt** gcggccgcat aatgcttaag
ctcctctagt acgtgt/**acta ctgcttcgaa** cgccggcgta ttacgaattc

2174 tcgaacagaa agtaatcgta ttgtacacgg ccgcataatc gaaattaata
agcttgtctt tcattagcat aacatgtgcc ggcgtattag ctttaattat

T7 promoter-2 lac operator

2224 cgactcacta taggggaatt gtgagcggat aacaattccc catcttagta
gctgagtgat atccccttaa cactcgccat ttgttaaggg gtagaatcat

Sequence of pETDuet-1-LIC1: 7425 bp

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