

Vector pCDFDuet-1-LIC1

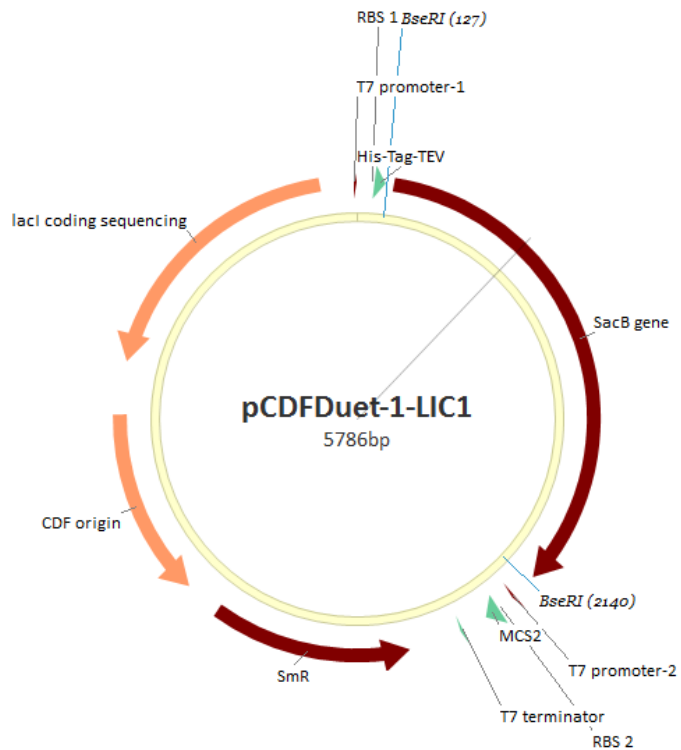
Updated in September 2015

Source	Constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	pCDFDuet-1-LIC1 is derived from pCDFDuet-1 (Novagen #71340-3) 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	Streptomycin (SmR)
Promoter	<i>T7lac</i> operator
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 Sac B 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' CAAGCT TCGTCATCA 3'
5' sequencing primer for pCDFDuet-1-LIC1	5' GGATCTCGACGCTCTCCCT 3'
3' sequencing primer for pCDFDuet-1-LIC1	5' GATTATGCGGCCGTGTACAA 3'

Location of features of pCDFDuet-1-LIC1:

- RBS-1: 58-63 bp
- His-Tag-TEV: 71-124 bp
- SacB gene: 141-2123 bp
- T7 promoter-2: 2219-2235 bp
- RBS-2: 2291-2296 bp
- MCS2: 2302-2437 bp
- T7 terminator: 2467-2514 bp
- Sm R (complementary): 2688-3476 bp
- CDF Origin (complementary): 3616-4354 bp
- Lac I coding sequencing (complementary): 4564-5655 bp
- T7 promoter-1: 5770-5786 bp

Map of pCDFDuet-1-LIC1:



pCDFDuet-1-LIC1 cloning/expression region

T7 promoter 1 → lac operator

5754 tcctgcatta ggaaattaat acgactcact ataggggaat tgtgagcgga
aggacgtaat cctttaatta tgctgagtga tatcccctta acactcgcct

rbs

18 taacaattcc cctgtagaaa taattttggt taactttaat aaggagatat
attgttaagg ggacatgttt attaaaacaa attgaaatta ttcctctata

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

68 accatgcatc atcatcatca tcacagcagc ggcagagaaa acttgatatt
tggtagtag tagtagtagt agtgtcgtcg ccgtctcttt tgaacataaa

Q G NdeI BseRI

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

BseRI 2x stop Hind III

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

2174 tcgaacagaa agtaatcgta ttgtacacgg ccgcataatc gaaattaata
agcttgtctt tcattagcat aacatgtgcc ggcgtattag cttaattat

T7 promoter 2 → lac operator

2236 cgactcacta taggggaatt gtgagcggat aacaattccc catcttagta
gctgagtgat atccccttaa cactcgccat ttgttaaggg gtagaatcat

Sequence of pCDFDuet-1-LIC1: 5786 bp

ggggaattgtgagcggataacaattcccctgtagaataatTTTgttTaaacttTaaataaggagatataccatgcatcatca
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