

Vector information sheet

Dated: 8th May 2013

Vector Name	pCDF-LIC
Source	Opher Gileadi
Sequence accession/link	

Description	<p>A DUET expression vector with two transcription units (each under a T7 promoter-Lac operator). The vector also carries the CloDF13-derived CDF replicon, <i>lacI</i> gene and streptomycin/spectinomycin resistance gene (SmR). This vector can be used in combination with pET vectors (including the pNIC series) and with pACYC vectors (including the pRARE plasmids in Rosetta strains).</p> <p>The first ORF is fused to a His₆ tag in 22-aa N-terminal tag, with a TEV protease cleavage site. Cloning into this site is done by LIC after cleaving the vector with BsaI; this removes a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose.</p> <p>It is possible to clone a second ORF using NdeI and XhoI restriction.</p> <p>Sequencing and colony PCR require specialized primers: do not use T7P.</p>
-------------	---

Antibiotic resistance	Spectinomycin, 50 µg/ml
ORF1	
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.	MHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.8 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	ACYCDuetUP1: GGATCTCGACGCTCTCCCT
3' sequencing primer	DuetDOWN1: GATTATGCGGCCGTGTACAA
ORF2	
Promoter	T7 - lacO
Cloning	NdeI - XhoI
Initiation codon	Supplied in PCR primer, within NdeI site
N-terminal fusion	none
C-terminal fusion	Possible S-tag, if no termination codon provided in PCR fragment.
Termination codons	supplied in PCR primer OR after S-tag.
Additional features	


```

2101                                     GCATTGACGG
                                         CGTAACTGCC

      BsaI          Lic3          BamHI
      ~~~~~ ~~~~~
2161 TCTCCAGTAA AGGTGGATAC GGATCCGGCC GCATAATGCT TAAGTCGAAC AGAAAGTAAT
      AGAGGTCATT TCCACCTATG CCTAGGCCGG CGTATTACGA ATTCAGCTTG TCTTTCATTA

      DuetUP1/DuetDN1 primers          T7 promoter          lacO
      ~~~~~ ~~~~~ ~~~~~
2221 CGTATTGTAC ACGGCCGCAT AATCGAAATT AATACGACTC ACTATAGGGG AATTGTGAGC
      GCATAACATG TGCCGGCGTA TTAGCTTTAA TTATGCTGAG TGATATCCCC TTAACACTCG

      lacO                                     NdeI
      ~~~~~ ~~~~~
2281 GGATAACAAT TCCCATCTT AGTATATTAG TTAAGTATAA GAAGGAGATA TACATATGGC
      CCTATTGTTA AGGGGTAGAA TCATATAATC AATTCATATT CTTCTCTAT ATGTATACCG
                                         XhoI
                                         ~~~~~
2341 AGATCTCAAT TGGATATCGG CCGGCCACGC GATCGCTGAC GTCGGTACCC TCGAGTCTGG
      TCTAGAGTTA ACCTATAGCC GGCCGGTGGC CTAGCGACTG CAGCCATGGG AGCTCAGACC
      S-Tag
      ~~~~~
      · G E T A A A K F E R Q H M D S S T S A A ·
2401 TAAAGAAACC GCTGCTGCGA AATTTGAACG CCAGCACATG GACTCGTCTA CTAGCGCAGC
      ATTTCTTTGG CGACGACGCT TTAAACTTGC GGTCTGTAC CTGAGCAGAT GATCGCGTCG
      · *
2461 TTAATTAACC
      AATTAATTGG

```

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.

pCDF-LIC sequence:

```

gccataaccgcaaagggttttgcgccattcgatgggtgtccgggatctcgacgctctcccttatgcgactc
ctgcattaggaaattaatacgcactcactataggggaattgtgagcggataaacaattcccctgtagaat
aatTTTgtTTaactTTaataaggagatataccatgcaccatcatcatcattcttctggtgtagatc
tgggtaccgagaacctgtacttccaatccatggagaccgacgtccacatataacctgccgttactatta
tttagtgaaatgagatattatgatattttctgaattgtgattaaaaaggcaactttatgccccatgcaac
agaaactataaaaaatacagagaaatgaaaagaaacagatagatttttttagttctttaggcccgtagtct
gcaaatccttttatgattttctatcaaacaaaagaggaaaatagaccagttgcaatccaaacgagagtc
taatagaatgaggtcgaaaagtaaatcgcgcggtttgttactgataaagcaggcaagacctaataatgt
gtaaagggcaaagtgtatactttggcgtcacccttacatatttttaggtcttttttattgtgcgtaac
taacttgccatcttcaaacaggagggtggaagaagcagaccgctaacacagtacataaaaaaggagac
atgaacgatgaacatcaaaaagtttgcaaaaagcaaacagtattaacctttactaccgcactgctggc
aggaggcgcaactcaagcgtttgcaaaagaaacgaacccaaaagccatataaggaaacatacggcatttc
ccatattacacgcatgatatgctgcaaatccctgaacagcaaaaaaatgaaaaatataaagttcctga
gttcgattcgtccacaattaaaaatatcttctgcaaaaaggcctggacgtttgggacagctggccatt
acaaaacactgacggcactgtcgcaactatcacggctaccacatcgtctttgacattagccggagatcc
taaaaatgcggtatgacacatcgatttacatgttctatcaaaaagtcggcgaaacttctattgacagctg
gaaaaacgctggccgctctttaaagacagcgcaaaattcgatgcaaatgattctatcctaaaagacca
aacacaagaatggtcagggtcagccacatttacatctgacggaaaaatccgtttattctacactgattt
ctccggtaaacattacggcaaaacaaactgacaactgcacaagttaacgtatcagcatcagacagctc
tttgaacatcaacggtgtagaggattataaatcaatctttgacggtgacggaaaaacgtatcaaatgt
acagcagttcatcgatgaaggcaactacagctcaggcgcaaacatacgtgagagatcctcactacgt
agaagataaaggccacaaatacttagtatttgaagcaaacactggaactgaagatggctaccaaggcga

```

agaatctttatTTTaaCAAagcatactatggCAAaagcacatcattcttccgtcaagaaagtcaaaaact
tctgCAAagcgataaaaaacgcacggctgagttagCAAacggcgctctcggtatgattgagctaaacga
tgattacacactgaaaaaagtgatgaaaccgctgattgcatctaacacagtaacagatgaaattgAACg
cgCGAACgtctTTTaaatgAACggCAAatggtacctgttcaactgactcccgcggtcaaaaatgacgat
tgacggcattacgtctAACgatatttacatgcttggttatgtttctaattctTTTaaactggccatacaa
gCGctgAACaaaactggccttggtTaaaaatggatcttgatcctaacgatgtaacctttacttactc
acacttCGctgtacctcaagcgaaaggaaacaatgtcgtgattacaagctatatgacaaacagaggatt
ctacgcagacaaacaatcaacgTTTgCGcctagcttccctgctgAACatcaaaggcaagaaaacatctgt
tgtCAAagacagcatccttgaacaaggacaattaacagTTaacaataaaaaacgcaaaagaaaatgccg
atctcctattggcattgacggctccagTaaaggTgatacggatccggccgataatgcttaagtcga
acagaaagTaatcgtattgtacacggccgataaatcgaaattaatacgactcactataggggaattgtg
agcggataacaattccccactcttagtatattagTTaagtataagaaggagatatacatatggcagatct
caattggatctcggccggccacgcgatcgtgacgtcggTaccctcgagtctggTaaagaaaccgctgc
TgCGaaattTgAACgCCagcacatggactcgtctactagcgcagcttaattaacctaggctgctgccac
cgctgagcaataactagcataacccttggggcctctaaacgggtcttgaggggtTTTTTgctgaaacc
tcaggcatttgagaagcacacggTcacactgcttccggtagtcaataaacggTaaaccagcaatagac
ataagcggctatTTTaaCGaccctgccctgAACcgacgaccgggtcatcgtggccggatctTgCGgcccc
tcggcttgaacgaattgTtagacattatTTTgCGactaccttggTgatctcgcctttcacgtagtgagac
aaattcttccaactgatctgCGcgcgagggcaagcgatcttcttctTgtccaagataagcctgtctagc
ttcaagTatgacgggctgatactgggCCggcagggcgtccattgccagtcggcagcgacatccttcgg
cgcgatTTTgCGgTtactgCGctgtaccaaagTcgggacaacgTaaagcactacatttcgctcatcgc
agcccagtcgggCGgagTtccatagcgtTaaaggTTcatttagcgcctcaaatagatcctgttcagg
aacggatcaaagagTtccctccgCGcgtggacctaccaaggcaacgctatgttctcttgcTTTTgtcag
caagatagccagatcaatgtcgatcgtggctggctcgaagatacctgcaagaatgtcattgCGctGCCa
ttctccaaattgcagTtCGcgttagctggataacGCCacggaatgatgtcgtcgtgcacaacaatggt
gacttctacagcCGgagaatctcgtctctccaggggaagccgaagTTTccaaaaggTcgttgatcaa
agctCGcCGcgtTgTTTTcatcaagccttacggTcacCGtaaccagcaaatcaatatcactgtgtggct
caggCGccatccactgCGgagccgtacaaatgTACggccagcaacgTcggTtcgagatggcgctcgat
gacGCCaactacctgatagTtagctgatacttcggcgatcaccgcttccctcactactcttccctttt
tcaatattattgaaGcatttatcagggttattgtctcactagcgcgatacatattTgcaatgtatttagaa
aaataacaaatagctagctcactcggTcgtcactcgcctccgggCGtgagactgCGgCGgCGctgCGgac
acatacaaaagtTaccacagatTccgTggataagcaggggactaacatgtgaggcaaaacagcagggcc
gCGcCGTggcgTTTTTccataggctccgccccctcctgccagagTtcaataaacagacgctTTTTcCGT
gcatctgTgggagcCGtgaggctcaaccatgaatctgacagTACgggCGaaacCCgacaggactTaaag
atccccaccgTTTTcCGgCGgTcgtccctctTgCGctctcctgttccgaccctgCCgTttaccggata
cctgttccgctTTTTctcccttacgggaagTgtggcgtTTTTctcatagctcacacactggTatctcggct
cggTgtaggTcgttCGctccaagctgggctgTaaGcaagaactccccgTtcagccgactgctgCGcct
tatccggTaaactgttcaactTgagTccaacCCgaaaagcACggtaaaacGCCactggcagcagccattg
gTaaactgggagTtcgCagaggattTgtTtagctaaacacgCGgtTgctctTgaaGTgTgCGcCAAagTc
CGgctacactggaaggacagatTtggtTgctgtgctctgCGaaagccagTtaccaggtTaaGcagTtC
cccaactgactTaaCcttcgatcaaaccacctccccaggtggTTTTTcgTttacagggcaaaagatta
cGCGcagaaaaaaggatctcaagaagatcctttgatctTTTtactgAACCGctctagatttcagTgc
aatttatctctTcaaatgtagcactgaagTcagccccatacgatataagTtgTaaTtctcatgttagt
catgccccCGccccaccggaaggagctgactgggtTgaaGGctctcaagggcatcggTcGagatccccg
TgCctaatgagTgagTaaactTacaTTaattgCGTtGCGctcactgcccGctttccagTcgggaaacct
gtCGTgCCagctgcattaatgaatCGGCCaACgCGCGgggagaggCGgtTtGCGatTgggCGccaggg
TggTTTTTctTTTcaccagTgagacgggcaacagctgattgCCctTcaccgCctggccctgagagatt
gCagcaagcggTccacgctggTTTTgccccagcagggcaaaatcctgtTtgatggTgTaaCGCGgga
tataacatgagctgtctcggTatcgtcgtatcccactaccgagatgtccgCaccaacgCGcagccCG
actCGgTaaTggCGcgcattTgCGcccagCGccatctgatcgtTggcaaccagcatcgcagTgggaacga
TgCctcattcagcattTgcatggTTTTgtTgaaaacCGgacatggcactccagTcgccttccCGTtccg
ctatCGgctgaattTgattgCGagTgagatattTatgCCagCCagCCagacgCagacgCGccgagacag
aactTaatgggccccgTaaacagcCGgattTgctggTgacccaatgCGaccagatgctccacgcccagTc
gCGTaccgTctTcatgggagaaaataaactgTtgatgggtgtctggTcagagacatcaagaaataacg
ccggaacattagTgcaggcagcttccacagcaatggcatcctggTcatccagCGgatagTtaatgatca
gcccactgacCGgtTgCGcGagaagattTgtgCaccgCCgctTtacaggcttcgacCGcctcgttcta
ccatCGacaccaccCGctggcaccagTtgatcggCGcGagatTtaatCGCCgCacaattTgCGacg
gCGcgtgCagggccagactggaggTggcaacGCCaatcagcaacgactgTttgCCCGccagTtgtTgtg
ccacCGggtTgggaatgTaaTcagctccGCCatCGCCgcttccactTTTTcCGCGTTTTcGcagaaa
cgtggctggcctggTtCaccacCGggaaacggTctgataagagacaccggcactctgCGacatcgt
ataacgTtactggTTTTcaccattcaccaccctgaattgactctcttccgggCGctatcat

