Vector information sheet

Dated: 8\textsuperscript{th} May 2013

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pCDF-LIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Opher Gileadi</td>
</tr>
<tr>
<td>Sequence accession/link</td>
<td></td>
</tr>
</tbody>
</table>

**Description**  
A DUET expression vector with two transcription units (each under a T7 promoter-Lac operator). The vector also carries the CloDF13-derived CDF replicon, lacI 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).  
The first ORF is fused to a His\textsubscript{6} 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.  
It is possible to clone a second ORF using NdeI and XhoI restriction.  
Sequencing and colony PCR require specialized primers: do not use T7P.

**Antibiotic resistance**  
Spectinomycin, 50 \( \mu \text{g/ml} \)

**ORF1**

<table>
<thead>
<tr>
<th>Promoter</th>
<th>T7 - lacO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning</td>
<td>LIC. (vector treated with BsaI, then with T4 DNA polymerase in presence of dGTP)</td>
</tr>
<tr>
<td>Initiation codon</td>
<td>Supplied in PCR primer</td>
</tr>
<tr>
<td>N-terminal fusion – seq.</td>
<td>MHHHHHSSGVDLGTENLYFQ<em>SM (</em> - TEV cleavage site)</td>
</tr>
<tr>
<td>N-terminal fusion – MW</td>
<td>2684.1 Da including Met (2465.8 Da removed by TEV cleavage)</td>
</tr>
<tr>
<td>Termination codons</td>
<td>supplied in PCR primer</td>
</tr>
<tr>
<td>Protease cleavage</td>
<td>TEV</td>
</tr>
<tr>
<td>Additional features</td>
<td></td>
</tr>
<tr>
<td>Preferred host</td>
<td>DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.</td>
</tr>
<tr>
<td>5’ sequencing primer</td>
<td>ACYCDuetUP1: GGTCTCGACGCTCTCCCT</td>
</tr>
<tr>
<td>3’ sequencing primer</td>
<td>DuetDOWN1: GATTATGCGGCCGTGACAA</td>
</tr>
</tbody>
</table>

**ORF2**

<table>
<thead>
<tr>
<th>Promoter</th>
<th>T7 - lacO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloning</td>
<td>NdeI - XhoI</td>
</tr>
<tr>
<td>Initiation codon</td>
<td>Supplied in PCR primer, within NdeI site</td>
</tr>
<tr>
<td>N-terminal fusion</td>
<td>none</td>
</tr>
<tr>
<td>C-terminal fusion</td>
<td>Possible S-tag, if no termination codon provided in PCR fragment</td>
</tr>
<tr>
<td>Termination codons</td>
<td>supplied in PCR primer OR after S-tag.</td>
</tr>
<tr>
<td>Additional features</td>
<td></td>
</tr>
</tbody>
</table>
Preferred host: DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.

5’ sequencing primer: DuetUP1: TTGTACACGGCCGATAATC

3’ sequencing primer: T7 terminator: GCTAGTTATTTGCTAGCGG

Polylinker region:

ACYCDuetUP1 primer

GCCATACGGC GAAAGGTTTT GCCGATCTCG ATGGTGTCGG GGATCGAC GCTCTCGAC
CGGTATGGCG CTTTCCAAAA CCGCTAGAAG TACACAGGC CCTAGAGCTG CGAGAGGGAA

T7 promoter: ~~~~~|~~~~lac operator~~

ATGCGACTCC TGCATTAGGA AATTAATACG ACTCACTATA GGGGAATTGT GAGCGGATAA
TACGAGG ACGTAATCCT TTAATTATGC TGAGTGATAT CCCCTTAACA CTCGCCTATT

---

CloDF13 ori

Spectomycin resistance

CloDF13 ori

pCDF-LIC

5717 bp

lacI

MCS-2 ORF

sacB

His6

TEV

Spectomycin resistance

NcoI

Lic3 (2179)

Lic5 (225)

NdeI (2335)

XhoI (2391)

BsaI (235)

BsaI (2166)

~~~

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

BsaI

~
Primers for LIC cloning:

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

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

pCDF-LIC sequence:

gccatacgcctgaaagtttgcgatcctctctcgcgcgatctctcctctctcatgtcgcgtcctccttatttccttgcttccttccttctcccccctctgctttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
