**Vector pETDuet-1-LIC1**  
*Updated in September 2015*

<table>
<thead>
<tr>
<th>Source</th>
<th>Constructed by Yanjun Li</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>Structural Genomics Consortium, Toronto</td>
</tr>
<tr>
<td>Description</td>
<td>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.</td>
</tr>
<tr>
<td>Antibiotic resistance</td>
<td>Amp R</td>
</tr>
<tr>
<td>Promoter</td>
<td>T7lac promoter</td>
</tr>
<tr>
<td>Cloning Methods</td>
<td>Insertion of DNA sequence into the 1st 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.</td>
</tr>
<tr>
<td>Initiation Codon</td>
<td>ATG site in vector at bp 71</td>
</tr>
<tr>
<td>N – terminal fusion sequence</td>
<td>MHHHHHHSNSRRENLYFQG</td>
</tr>
<tr>
<td>Termination codons</td>
<td>TGATGA included in 3’ PCR primer addition</td>
</tr>
<tr>
<td>5’ primer addition for amplification of insert 1</td>
<td>5’ TTGTATTTCCAGG 3’</td>
</tr>
<tr>
<td>3’ primer addition for amplification of insert 1</td>
<td>5’ CAAGCTTCGTCATCA 3’</td>
</tr>
<tr>
<td>5’ sequencing primer for pETDuet-1-LIC1</td>
<td>5’ ATGCGTCCGCGTAG 3’</td>
</tr>
<tr>
<td>3’ sequencing primer for pETDuet-1-LIC1</td>
<td>5’ GATTATGCGGCCGTGACAA 3’</td>
</tr>
</tbody>
</table>
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**

T7 promoter-1

```plaintext
7394 tcgatccgcc gaaattaata gcactcacta taaggggaat tggtagcgga agcttagggcgc ctttaattat gctgagtgat atccccctta acactcgccct

```

**lac operator**

lac operator

```plaintext

```

**rbs**

rbs

```plaintext

```

<table>
<thead>
<tr>
<th>M</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>H</th>
<th>S</th>
<th>S</th>
<th>G</th>
<th>R</th>
<th>E</th>
<th>N</th>
<th>L</th>
<th>Y</th>
<th>F</th>
</tr>
</thead>
</table>

```plaintext

18 taacaattccc cctctagaaaa taatattgtt taacttttaag aaggagatat attgttaagg ggagatcttt attaaaacaa attgaaatc ttcccttata

```

**Q**

Q

```plaintext

```

**G**

G

```plaintext

```

**NdeI**

NdeI

```plaintext

```

**BseRI**

BseRI

```plaintext

```

<table>
<thead>
<tr>
<th>BseRI</th>
<th>2x stop</th>
<th>HindIII</th>
</tr>
</thead>
</table>

```plaintext

2124 gaggagatca tcgaca/tgat gacgaagctt gcggcgcgcat aatgcttaag ctcctctagt acgtg/tacta ctccttcgaa cgccgcgcta ttacgaattc

```

2174 tcgaacagaa agtacgta ttgtagcaggg ccgcataatc gaaattaata agcttgcttt ttcattaggac aacatgtgcc ggctattgat atttatata

**T7 promoter-2**

T7 promoter-2

```plaintext

```

**lac operator**

lac operator

```plaintext

```

```plaintext

2224 gcactcacta taggggaatt tggagcggag aacaattccc cattttagta gctgagtgat atcccccttac cactcgccct ttgtaaggg gtagaatcct

```

```plaintext

```
Sequence of pETDuet-1-LIC1: 7425 bp

gtgatgagcggcagaaactgccatgcagccaggtttcttgcaggaatctctgtgaggaataaacgagagtttcatcagcctgagttttctctc
gagcagcatcagcagcggcagagaaaacttgtatttttccagggccatatgagttctcctgaaagatccataacttcgtatagcatacattatc
gacgctcaacccatcataatttttttattttttattttttttttattttttatttttttttttttttttttttttttttttttttttttttttttttattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt