# pET28-Cub Vector

<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>
</tbody>
</table>

## Description
The pET28-Cub vector was derived from expression plasmid pET28-MHL (made by Peter Loppnau, SGC). It is designed for T7 promoter driven co-expression of recombinant proteins with ubiquitin located at C-terminal. The C-terminal ubiquitin is preceded by the ribosomal binding site (RBS) and followed by two stop codons. The pET28-Cub keeps the features of pET28-MHL as addition of 18 amino acid N-terminal fusion tag containing 6X His followed by a TEV cleavage site as well as removing of the GSS residues after the Met start site to reduce N-terminal gluconoylation via preventing N-terminal Met excision.

## Antibiotic resistance
Kanamycin, 50 ug/ml

## Promoter
T7 - lacO

## Cloning Methods
Insertion of DNA sequence into the cloning/expression region is preformed 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.

## N-terminal fusion sequence
MHHHHHHSSGRENLYFQG

## Termination codons
TGATGGA is right downstream of ubiquitin located at C-terminal of this vector.

## Primer mix for screening
T7- forward and reverse mix

<table>
<thead>
<tr>
<th>5’ primer for amplification of insert</th>
<th>5’ TTGTATTTCCAGGCC 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ primer for amplification of insert</td>
<td>5’TATCTCCTTCTTTTA 3’</td>
</tr>
<tr>
<td>5’ sequencing primer T7-Fwd</td>
<td>5’ AATTAATACGACTCATATAGGG 3’</td>
</tr>
<tr>
<td>3’ sequencing primer T7-Rev</td>
<td>5’ ATGCTAGTTATTGCTAGCGG 3’</td>
</tr>
</tbody>
</table>
**pET28-Cub vector map:**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7 promoter</td>
<td>4985-4980</td>
</tr>
<tr>
<td>N-terminal tag</td>
<td>5071-5124</td>
</tr>
<tr>
<td>N-terminal cloning site</td>
<td>5109-5123</td>
</tr>
<tr>
<td>C-terminal cloning site</td>
<td>7139-7153</td>
</tr>
<tr>
<td>T7 terminator</td>
<td>7481-7528</td>
</tr>
<tr>
<td>f1 origin</td>
<td>12-466</td>
</tr>
<tr>
<td>KanR coding sequence</td>
<td>563-1374</td>
</tr>
<tr>
<td>pBR322 origin</td>
<td>2087</td>
</tr>
<tr>
<td>lacI coding sequence</td>
<td>3517-4596</td>
</tr>
<tr>
<td>SacB coding sequence</td>
<td>5140-7122</td>
</tr>
</tbody>
</table>
pET28-Cub cloning/expression region:

**T7 FWD**

```
4967  ctgcatcccg  cgaattaat  agcactcact  atagggaat  tggagcggga
       gagctagggc  gctttaatta  tgctgagtga  tatcccttta  acactcgct
```

**lac operator**

```
5017  taacaatccc  cctctagaaaa  taacttttaag  aagagatat
       attgttaagg  ggagatctttt  attaaacaaa  attgaatct  ttctcttata

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

**5’ addition**

```
5067  accatgcatc  atcatcatca  tcacagcgc  ggcaagaaa  actttgtattt
       Tgtagtagttagttagtactgctgcg  ccgctctcttt  tgaacataaa

Q G  NdeI  BseRI
```

```
5117  ccagggc/cat  atgagtt  ctcctc-----SACB  cassette(1983bp)----
       Ggtcccg/gta  tactcaag  gaggag"'

BseRI  RBS
```

```
7123  gagagatca  tgcaca/TAAAAGAAGGAGATATACCGAGATAGAAAAAGAGTTGCTGCGTGCACTGCTG/ubiquitin(228bp)/TGATGA
       ctcctctagt  acgtgt/ATTTTCCTCTCTATATGG/ubiquitin(228bp)/ACTACT
```

**3’ addition**

```
7392  tcgagcacca  ccaccaccac  cactgagatc  cggctgctaa  caaagccccga
```

**T7 REV**

```
7442  aaggaagctg  agttggctgc  tgccaccgct  gagaataac  tagcataaac
```