pCW-LIC Vector (GenBank accession EF460848)

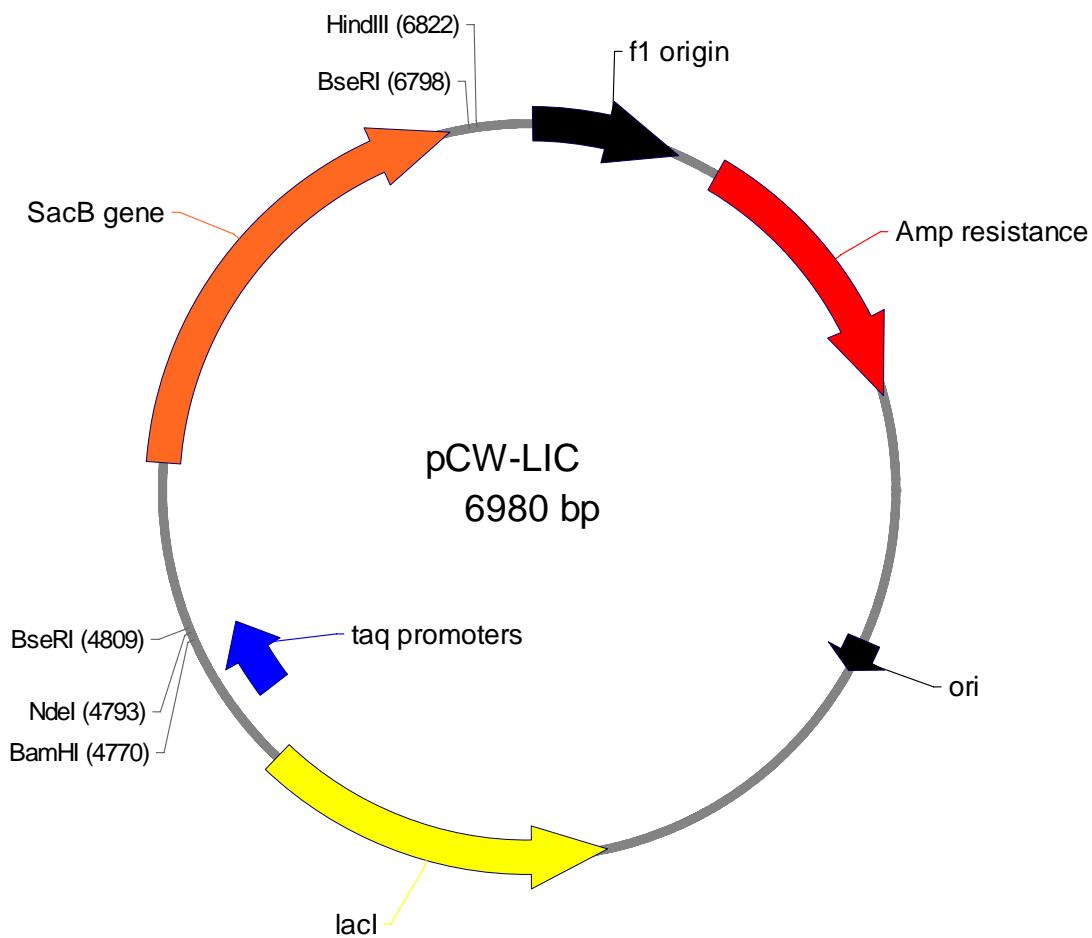|	
Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pCW-LIC vector was derived from expression plasmid pCWori$^+$ [Prof.Frances Arnold's Lab]. It is used for tac promoter driven expression of cloned genes such as Cytochrome P450s in *E. coli* without fusion tag sequences coded by the vector. Fusion tags are added to inserts using PCR primers. Two stop codons are included in the vector at the C-terminal cloning site.

Antibiotic resistance	Ampicillin, 100 ug/ml
Promoter	Three sequential tac promoters, 2nd two are active
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.
Initiation Codon	NdeI site in vector at 4796 bp
N – terminal fusion sequence	none
Termination codons	TGATGA included in 3' PCR primer and vector cloning site. No amino acid residues added at cloning junction
Additional features	
Prefered Hosts	
5' primer for amplification of insert	5' TTA GGA GGT CAT ATG--- 3'
3' primer for amplification of insert	5' TAA GCT TCG TCA TCA--- 3'
5' sequencing primer pCW-SEQ-fwd	5, ACATCGTATAACGTTACTGG 3' or 5' GATGCTTAGGAGGTCATATG 3'
3' sequencing primer pCW-rev	5' CTTTCGTCTTCAAGCAGATCTG 3'

pCW-LIC vector map

double Ptac promoter	4514-4770
N-terminal cloning site	4784-4798
C-terminal cloning site	6814-6828
f1 origin	12-467
bla coding sequence	597-1454
pBR322 origin	2215
lacI coding sequence	3252-4331
sacB coding sequence	5320-6738

HindIII (6822)

BseRI (6798)

f1 origin

SacB gene

Amp resistance

pCW-LIC
6980 bp

BseRI (4809)

taq promoters

NdeI (4793)

BamHI (4770)

ori

lacI

pCW-LIC cloning/expression region

```
4583   actccccatc cccctgttga caattaatca tcggctcgta taatgtgtgg
       tgaggggtag ggggacaact gttaattagt agccgagcat attacacacc


                                                   BamHI        ..............
4633   aattgtgagc ggataacaat ttcacacagg aaacaggatc catcgatgct
       ttaacactcg cctattgtta aagtgtgtcc tttgtcctag gtagctacga


       pCW-Seq-fwd
       ........................................▶
               NdeI Met              BseRI
4778   taggaggtcat atg/attatga gttctcctc--SACB cassette(2 kb)-
       atcctccagta tac/taatact caagaggag


       BseRI               Stop     HindIII
6791   gaggagatca tgcaca/tgat gacgaagctt atcgatgata agctgtcaaa
       ctcctctagt acgtgt/acta ctgcttcgaa tagctactat tcgacagttt


                                               pCW-rev
                                         ◀..............................
6841   catgagcaga tctgagcccg cctaatgagc gggctttttt ttcagatctg
       gtactcgtct agactcgggc ggattactcg cccgaaaaaa aagtctagac


       .......................................................
6891   cttgaagacg aaagggcctc gtgatacgcc tatttttata ggttaatgtc
       gaacttctgc tttcccggag cactatgcgg ataaaaatat ccaattacag
```