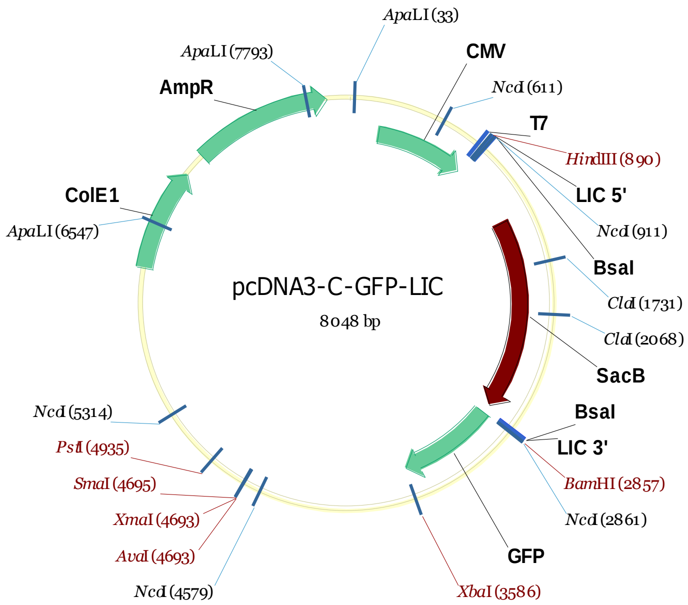
Vector information sheet

Dated: 8th May 2013

Vector Name	**pCDNA3-C-GFP-LIC**
Source	Grazyna Kochan
Sequence accession/link	(SGC)

Description	pCDNA3 eukaryotic expression vector with GFP tag as C-terminal fusion peptide. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose

Antibiotic resistance	Ampicillin, 100 μg/ml
Promoter	CMV
Cloning	LIC. (vector treated with BsaI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	SKGGYGSMVSKGEELFTGVVPILVELDGDVNGHKFS VSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLTY GVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKD DGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHK LEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQL ADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKR DHMVLLEFVTAAGITLGMDELYK
C-terminal fusion – MW	27578.14 Da
Termination codons	Present in vector
Protease cleavage	no
Additional features	
Preferred host	Mammalian cell lines (HEK HeLa, BHK BSC 1etc.
5' sequencing primer	pcDNA3-fwd (50), and T7fwd. TCCAAAATGTCGTAACAACTCC
3' sequencing primer	pcDNA3-rev (48): TTTTATTAGGAAAGGACAGTGG



Polylinker region:

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                                                       T7
                                          ~~~~~~~~~~~~~~~~~~~~~
                 CMV                                                                 LIC 5'
     ~~~~~~~~~~~~~~~~~~~~~~~~~                                                       ~
                                                                         HindIII
                                                                         ~~~~~~~
 841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGATACCCAA GCTTACCTGT
     GACGAATGAC CGAATAGCTT TAATTATGCT GAGTGATATC CCTATGGGTT CGAATGGACA
                      BsaI
                     ~~~~~~~
         LIC 5'
     ~~~~~~~~~~~~~~~
               NcoI
              ~~~~~~~
 901 ACTTCCAATC CATGGAGACC GACGTCCACA TATACCTGCC GTTCACTATT ATTTAGTGAA
     TGAAGGTTAG GTACCTCTGG CTGCAGGTGT ATATGGACGG CAAGTGATAA TAAATCACTT

------------------------------SacB---------------------------------------

                     BsaI
                    ~~~~~~
                              LIC 3'                            GFP
                          ~~~~~~~~~~~~~~~~~           ~~~~~~~~~~~~~~~~~~~~
                                                    NcoI
                                                   ~~~~~~~
                                               BamHI
                                               ~~~~~~~
                                                         M  V  S   K  G  E  E ·
2821 TATTGGCATT GACGGTCTCC AGTAAAGGTG GATACGGATC CATGGTGAGC AAGGGCGAGG
     ATAACCGTAA CTGCCAGAGG TCATTTCCAC CTATGCCTAG GTACCACTCG TTCCCGCTCC
                              GFP
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  L  F  T   G  V  V   P  I  L  V   E  L  D   G  D  V   N  G  H  K ·
2881 AGCTGTTCAC CGGGGTGGTG CCCATCCTGG TCGAGCTGGA CGGCGACGTA AACGGCCACA
     TCGACAAGTG GCCCCACCAC GGGTAGGACC AGCTCGACCT GCCGCTGCAT TTGCCGGTGT
                              GFP
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  F  S  V   S  G  E   G  E  G  D   A  T  Y   G  K  L   T  L  K  F ·
2941 AGTTCAGCGT GTCCGGCGAG GGCGAGGGCG ATGCCACCTA CGGCAAGCTG ACCCTGAAGT
     TCAAGTCGCA CAGGCCGCTC CCGCTCCCGC TACGGTGGAT GCCGTTCGAC TGGGACTTCA
                              GFP
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     ·  I  C  T   T  G  K   L  P  V  P   W  P  T   L  V  T   T  L  T  Y ·
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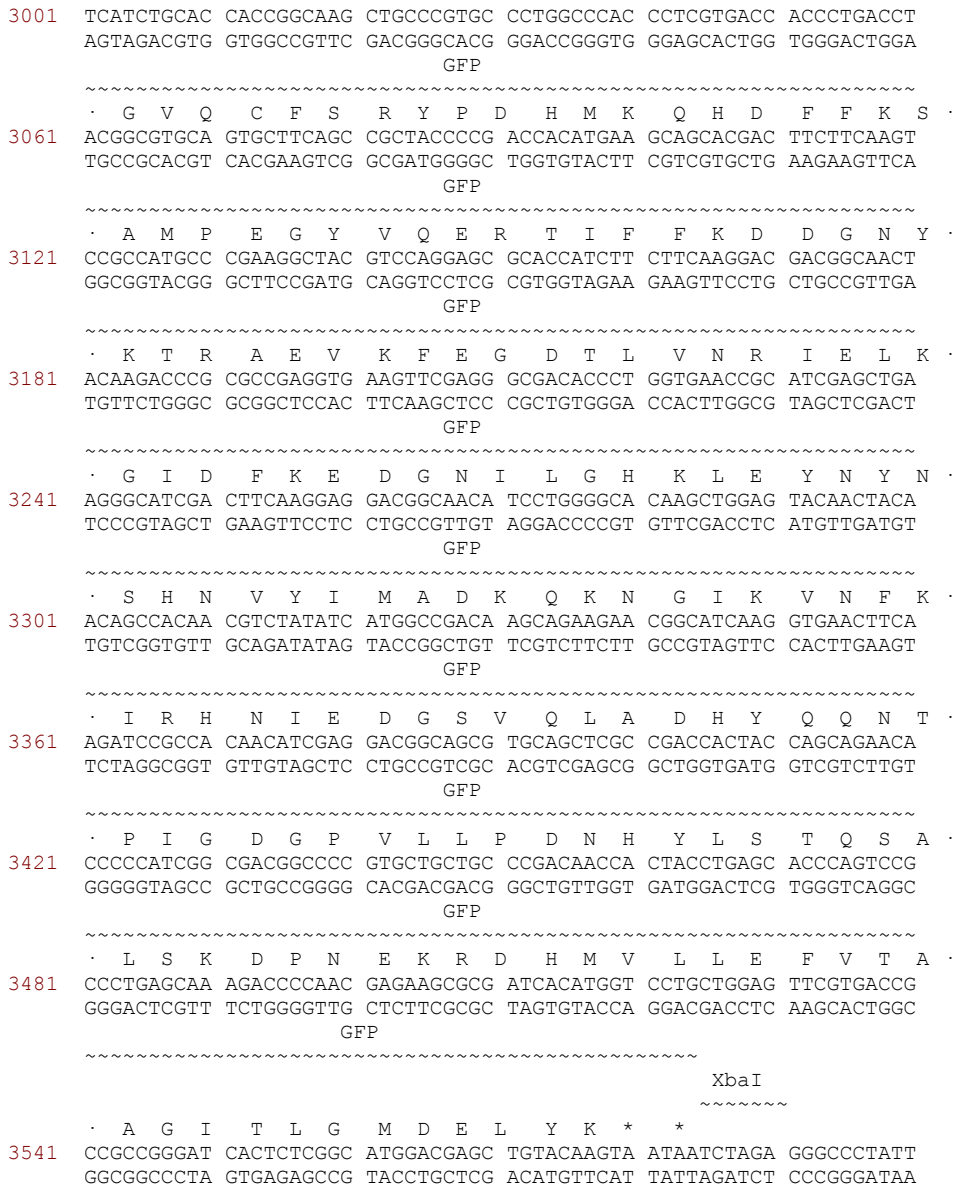

Primers for LIC cloning:

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

Downstream: add TATCCACCTTTACTGGA to 5' end of downstream primer;

pcDNA3-GFP-LIC sequence:

gacggatcgggagatctcccgatcccctatggtgcactctcagtacaatctgctctgatgccgcatagt
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