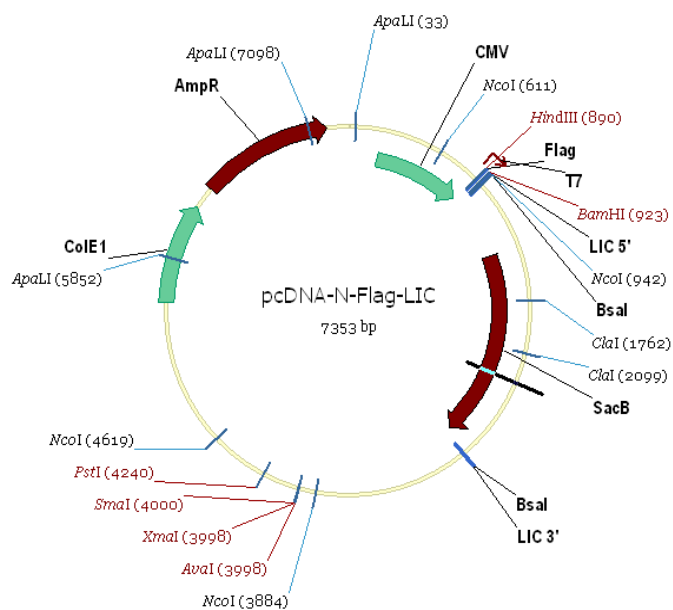


Vector information sheet

Dated: 8th May 2013

Vector Name	pCDNA3-N-Flag-LIC
Source	Grazyna Kochan
Sequence accession/link	(SGC)
Description	Mammalian expression vector with Flag tag as N-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
N-terminal fusion – seq.	MDYKDDDDKGSLYFQSM (Met-from cloned gene)
N-terminal fusion – MW	2058.2 Da including Met
Termination codons	supplied in PCR primer
Protease cleavage	no
Additional features	
Preferred host	Mammalian cell lines (HEK, HeLa, BHK, BSC1, etc.)
5' sequencing primer	pcDNA3_N-Flag-fwd (50): TCCAAAATGTCGTAACAACCTCC
3' sequencing primer	pcDNA3-rev (48): TTTTATTAGGAAAGGACAGTGG



Polylinker region:

1351 TTTGAGATGA GGATAAAAATA CTCTGAGTCC AAACCGGGCC CCTCTGCTAA
pcDNA3_N-Flag-fwd

1401 CCATGTTTCAT GCCTTCTTCT TTTTCCTACA GTCCTGGGC AACGTGCTGG

1451 TTTGTGCTGT CGACCCCAAG CTTATGGACT ACAAAGACGA TGACGACAAG
M D Y K D D D D K
HINDIII

1501 G S L Y F Q S M
GGATCCCTGT ACTTCCAATC CATGGAGACC GACGTCCACA TATACCTGCC
LIC 5' BSAI

3431 SacB linker - TATTGGCATT GACGGTCTCC
BSAI

3451 AGTAAAGGTG GATACGGATC TAGAACTAGT AACGGCCGCC
LIC 3'

Primers for LIC cloning:

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

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

pcDNA3-N-Flag-LIC sequence:

gacggatcgggagatctcccgatcccctatggtgcactctcagtacaatctgctctgatgccgcatagt
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