

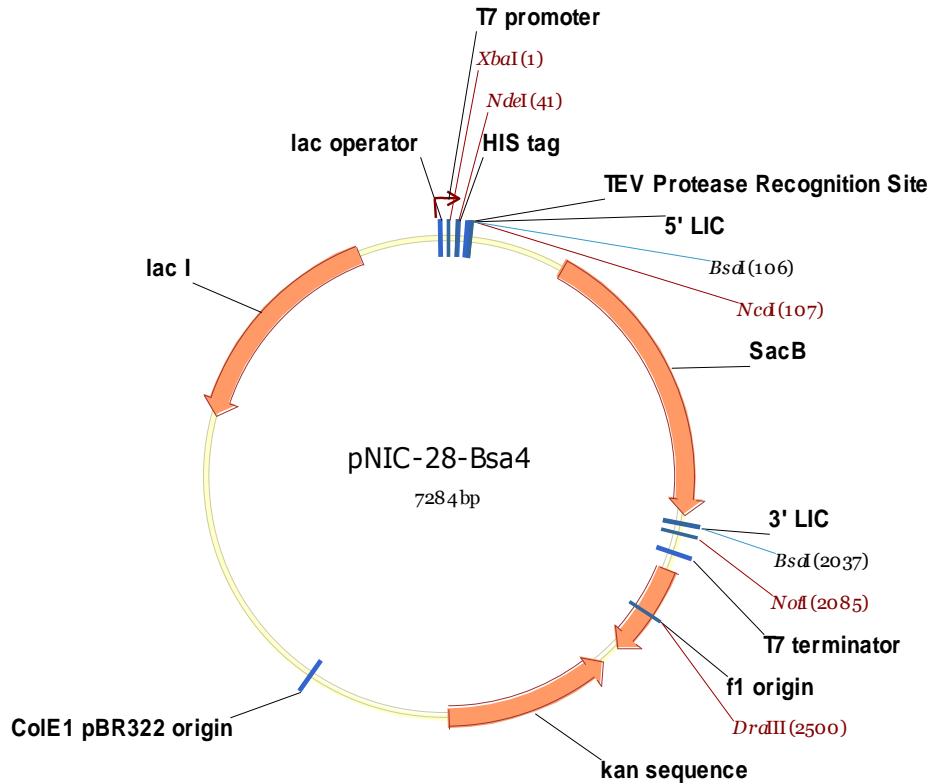
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC28-Bsa4</b>
Source	Opher Gileadi
Sequence accession/link	Genebank <a href="#">EF198106</a>

Description	pET expression vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	--

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.8 Da removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



### Polylinker region:

T7-forward	pLIC-forward
-----→	-----→
	lac operator
~~~~~	
7222 CTCGATCCCG CGAAATTAAT ACGACTCACT ATAGGGGAAT TGTGAGCGGA TAACAATTCC GAGCTAGGGC GCTTTAATTA TGCTGAGTGA TATCCCCTTA ACACTCGCCT ATTGTTAAGG	NdeI ~~~~~
M H H H H H .	
7282 CCTCTAGAGAA TAATTTGTT TAACTTAAG AAGGAGATAT ACATATGCAC CATCATCATC GGAGATCTTT ATTAAAACAA ATTGAAATTC TTCCCTCTATA TGTATACGTG GTAGTAGTAG	
Upper-LIC BsAI	
~~~~~ -----	
58 · H S S G V D L G T E N L Y F Q S ATCATTCTTC TGGTGTAGAT CTGGTACCG AGAACCTGTA CTTCCAATCC ATGGAGACCG TAGTAAGAAG ACCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC	
..... (SacB fragment) .....	
118 ACGTCCACAT ..... TGCAGGTGTA	EcoRI
BsAI Lower-LIC BamHI ~~~~~ SacI	
~~~~~ ~~~~~ ~~~~~ ~~~~~	
2010 GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTGAGCTC CTATAGGATA ACCGTAAGT CCAGAGGTCA TTTCCACCTA TGCCTAGGCT TAAGCTCGAG	
SalI HindIII	
***** ~~~~~	
2070 CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACAC CACCACTGAG ATCCGGCTGC GCAGCTGTTC GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGAAC TCAGGCCGACG	
T7-reverse	
←-----	

2130 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCAT  
ATTGTTTCGG GCTTCCCTTC GACTCAACCG ACGACGGTG CGACTCGTTA TTGATCGTAT  
←-----  
pLIC-rev

### Primers for LIC cloning:

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

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

### pNIC28-Bsa4 sequence:

ctgaaaatttgttaacttaagaaggagatatacatatgcaccatcatcatcatcatttttcgt  
tgtagatctgggtaccgagaacctgtacttccaatccatggagaccgacgtccacatataccgt  
tcactattatttagtgaardatgatattatgatatttctgaattgtgattaaaaaggcaacttatgc  
ccatgcaacagaaactataaaaaatacagagaatgaaaagaaacagatagatttttagttcttaggc  
ccgtagctgcaatcctttatgatttctatcaaacaagaaagaggaaatagaccagttgcaatccaa  
acgagagtctaatacgatgaggtcgaaaagtaatcgccggttggttactgataaagcaggcaagac  
ctaaaatgtgtaaaggcaaaagtgtatacttggcgtcacccttacatatttaggtcttttatt  
gtgcgtaactaacttgcatttcaaacaggaggctggaagaagcagaccgctaacacagtacataaa  
aaaggagacatgaacgatgaacatcaaaaagttgcaaaacaagcaacagtattaccccttactaccgc  
actgctggcaggaggcgcaactcaagcgttgcgaaagaaacgaaaccaaaagccatataaggaaacata  
cgccattccatattacacgccccatgtatgcataatccctgaaacagcaaaaaatgaaaatataaa  
agttcctgagttcgattcgccacaattaaaaatatcttctgcaaaaggcctggacgttggagag  
ctggccattacaaaacactgacggcactgtgcaactatcacggctaccacatcgtcttgattagc  
cgagatcctaaaaatgcggatgacacatcgatttacatgatcttcaaaaagtcggcggaaacttctat  
tgacagctgaaaaacgctgcccgtttaaagacagcgcacaattcgatgcaatgatttattct  
aaaagaccaacacaagaatggtcaggttcagccacattacatctgacggaaaaatccgttattct  
cactgatttctccggtaaacattacggcaaaacactgacaactgcacaagttacgtatcagcatc  
agacagctttaacatcaacggttagaggattataatcaatcttgcacggtagggaaaaacgta  
tcaaaatgtacagcagttcatcgatgaaggcaactacagctcaggcgacaaccatacgctgagagatcc  
tcactacgtagaagataaaggccacaaatacttagtatttgaagcaacactggaactgaagatggcta  
ccaaggcagaatctttattaacaaacgatacatatggcaaaagcacatcatttccgtcaagaaag  
tcaaaaacttctgcaagcgataaaaaacgcacgctgagtttagcaacaggcgtctcggtatgattga  
gctaaacgatgattacacactgaaaaaagtgtatgaaaccgctgattgcatacacaactgatgaa  
aattgaacgcgcgaacgtctttaaattgaacggcaatggatcgatcttactgactcccgccatcaaa  
aatgacgattgacggcattacgtctaacgatatttacatgatcttgcattttcttaactgg  
cccatacaagccgtgaacaaaactggcctgtttaaattggatcttgcatttcaacgatgtac  
tacttactcacacttcgttacctaagcgaaggaaacaatgtcgatcttgcatttgcatttgcatt  
cagaggatttacgcagacaacaatcaacgcttgccttagcttgcatttgcatttgcatttgcatt  
aacatctgttgcatttgcatttgcatttgcatttgcatttgcatttgcatttgcatttgcatt  
aaaatgccgatatttttttttttttttttttttttttttttttttttttttttttttttttttttt  
gtcgacacttgcggccgcactcgagaccaccaccaccactgagatccggctgctaacaagcc  
cgaaaggaaagctgagttggctgctgcccaccgctgagcaataactagcataacccttggggccct  
cggttcttgagggttttttttttttttttttttttttttttttttttttttttttttttttttt  
gcggccgcatattaaagcgcggcggtgtgggttacgcgcagcgtgaccgctacacttgc  
cgccccgtccttgccttctcccttcttcgttgcacgttgcggccgttcccccgtcaagctctaa  
atcgggggctccctttagggttccgatttagtgcatttgcggccatcgaccccaaaaaacttgc  
gtgatggttcacgttagtggccatgccttgcatttgcggccatcgatagcgttttgc  
tcttaatagtggactcttgcatttgcatttgcggccatcgatagcgttttgc  
tataaggatatttgcgatttgcctattggtaaaaaatgagctgatatttgc  
atttacaaaatattaacgatatttgcggccatcgatagcgttttgc  
tttgcggccatcgatatttgcggccatcgatagcgttttgc  
agcatcaaactgcaatttgcatttgcggccatcgatagcgttttgc  
tgtaatgaaggagaaaactcaccgaggcagttccataggatggcaagatc  
ccgactcgtccaacatcaatacacaaccttatttgc  
tcaccatqagtgacqactgaatccggqgagaatggcaaaagttatgc  
atttgcatttccagactgttca

acaggccagccattacgctcgtcatcaaaaatcactcgcatcaaccaaaccgttattcattcgtgattgc  
gcctgagcgagacaaatacgcgatcgctgttaaaaggacaattacaacaggaatcgaatgcacccgg  
cgcaggaacactgccagcgcatcaacaatatttcacctgaatcaggatattctctaatacctggaat  
gctgtttccgggatcgcaagtggtagtaaccatgcacatcaggatcaggataaaatgcttgatg  
gtcggaaagaggcataaattcgtcagccagtttagtctgaccatctcatcgtaaacatcattggcaacg  
ctaccttgcacatgtttcagaaacaactctggcgcatcggtttccatcataatcgatagattgtcgca  
cctgattgcccacattatcgcaagccattataccatataatcagcatccatgttggaaatttaat  
cgcggcctagagcaagacgttccgttaatatggctcataacaccctgttattactgtttatgtaa  
gcagacagtttattgttcatgacaaaatccctaactgttagttttcgactgtttccactgaccc  
cgtagaaaagatcaaaggatcttgcagatccttttgcgcgtaatctgtgttgcgttgcaccc  
aaaaccaccgcgtaccagcgggtttgttgcggatcaagagactaccactctttccgaaggtaac  
tggcttcagcagagcgcagataccaaatactgtcctctagtgttagccgttagttaggcccaccactcaa  
gaactctgttagcaccgcctacatacctcgctctgctaaccgttaccactgtggctgtccactgtggcga  
taagtctgttaccgggttgcactcaagacgatagttaccggataaggcgcagcggctggctgaac  
gggggggttgcacacagccagcttggagcgaacgacccactacccgaactgagatacactacgcgtga  
gctatgagaaagcgcacgcgttccgaaggagaaaaggcggacaggatccgttaagcggcagggtcgg  
aacaggagagcgcacgcggggacttccaggggaaacgcctgttatcttatacgatcgttgcgggttgc  
ccacctctgacttgcgtcgtatcttgcgttatctgttgcgtcaggggggggggggactatggaaaaacgc  
caacgcggccttttacggtcctggcctttgcgttgcgttatgcgttatgcgttgcgttgcgttgc  
ccctgattctgtgataaccgttattaccgccttgcgttatgcgttatgcgttatgcgttgcgttgc  
cgagcgcagcgtcgttgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gtcggttgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
cgtatatacactccgcatacgactgtgactgggtcatggctgcgcggacacccccaacaccgc  
acgcgcctgcacgggcttgcgttgcgttgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gcatgtgtcagaggttttaccgtcattaccgaaacgcgcgaggcagctgcgttgcgttatgcgttatgc  
gtcggttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ttaatgtctggctctgataaaagcgggcatgttaaggcgggttttgcgttatgcgttatgcgttatgc  
ccgtgttaaggggatttgcgttatggggtaatgataccgtgaaacagagagaggatgcgtcacgatac  
gggttactgtatgtatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ggcgggaccagagaaaaatcactcagggtcaatgcgcgttatgcgttatgcgttatgcgttatgc  
aggtagccagcgcacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ccagactttacgaaacacggaaaccgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
agcgtcgcttcacggttcgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ctagccgggtcctcaacgcacaggacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
cctgcttcgcggaaacgtttggcggaccagtgcgttatgcgttatgcgttatgcgttatgcgttatgc  
cgttataccgcacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
agagcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
tcatgcggccaccgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gtgcctaatgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
tgtcgccagctgcattatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gtggttttctttcaccagtgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
tgcagcaagcggccacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
atataacatgatgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gactcggttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
atgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gctatcggttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gaacttaatggcccgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
cgcgttacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gccggaaacattatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
agcccaactgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
accatgcacaccaccacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ggcgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gccacgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
acgtggctggctggttcaccacgcggaaacggcttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
tataacgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
cgaaagggtttgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gaagcagccagtagtaggttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ggcgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gaagtggcgagccgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
gccgggttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgcgttatgc  
ctcactatagggaaattgtgagcggataacaattccct

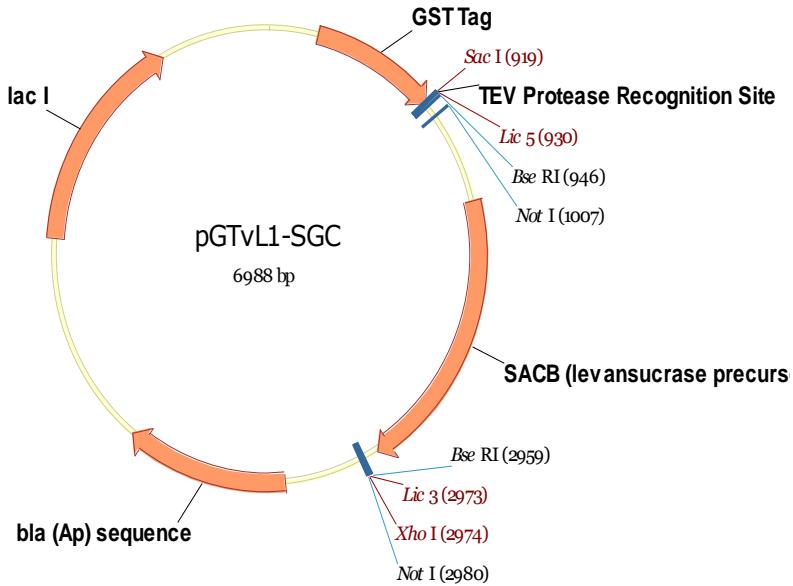
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pGTvL1-SGC</b>
Source	Jonathan Elkins (SGC, Oxford)
Sequence accession/link	(SGC)

Description	pGEX expression vector with N-terminal GST tag and TEV protease cleavage site. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose.
-------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	ampicillin
Promoter	Tac promoter (lac/IPTG inducible)
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	(readthrough from GST gene).
N-terminal fusion – seq.	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGAVLDIYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSSSENLYFQ*S(M) (* - TEV cleavage site)
N-terminal fusion – MW	26752.6 including Methionine (26534.3 removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV protease
Additional features	
Preferred host	Many E. coli strains (not dependent on T7 RNA polymerase)
5' sequencing primer	pGEX-5': GGGCTGGCAAGCCACGTTGGTG
3' sequencing primer	pGEX-3': CCGGGAGCTGCATGTGTCAGAGG



### Polylinker region:

		GST protein.....>																											
241	CACAGGAAAC	AGTATTTCATG	TCCCCTATAAC	TAGGTTATTG	GAAAATTAAG	GGCCTTGTGC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	P T R L L	L E Y L E	E K Y E E H	L Y E R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
301	AACCCACTCG	ACTTCTTTG	GAATATCTTG	AAGAAAAATA	TGAAGAGCAT	TTGTATGAGC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	D E G D K W	R N K K F	E L G L E	F P N L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
361	GCGATGAAGG	TGATAAATGG	CGAAACAAAAA	AGTTTGAATT	GGGTTGGAG	TTTCCAATC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	P Y Y I D G	D V K L T Q S	M A I I R Y I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
421	TTCCTTATTA	TATTGATGGT	GATGTTAAAT	TAACACAGTC	TATGCCATC	ATACGTTATA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
.	A D K H N M	L G G C P K E	R A E I S M L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
481	TAGCTGACAA	GCACAACATG	TTGGGTGGTT	GTCCAAAAGA	GCGTGCAGAG	ATTCAATGC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
.	E G A V L D	I R Y G V S R	I A Y S K D F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
541	TTGAAGGAGC	GGTTTGGAT	ATTAGATACG	GTGTTTCGAG	AATTGCATAT	AGTAAAGACT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
.	E T L K V D	F L S K L P E	M L K M F E D	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
601	TTGAAACTCT	CAAAGTTGAT	TTTCTTAGCA	AGCTACCTGA	AATGCTGAAA	ATGTTCGAAG	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
.	R L C H K T	Y L N G D H V	T H P D F M L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
661	ATCGTTATG	TCATAAAACA	TATTTAAATG	GTGATCATGT	AACCCATCCT	GACTTCATGT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
.	Y D A L D V	V L Y M D P M	C L D A F P K	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
721	TGTATGACGC	TCTTGATGTT	GTTTTATACA	TGGACCAAT	GTGCCTGGAT	GCGTTCCCAA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.				
.	L V C F K K	R I E A I P Q	I D K Y L K S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
781	AATTAGTTG	TTTTAAAAAA	CGTATTGAAG	CTATCCCACA	AATTGATAAG	TACTTGAAAT	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
.	S K Y I A W	P L Q G W Q A	T F G G G D H	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.						
841	CCAGCAAGTA	TATAGCATGG	CCTTGCAGG	GCTGGCAAGC	CACGTTGGT	GGTGGCGACC	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
TEV protease cleavage ↓																													
901	P P K S S S	E N L Y F Q S																											
	ATCCTCCAAA	ATCGAGCTCA	GAGAACCTGT	<b>ACTTCCAATC</b>	<b>CATAAGCTAG</b>	<u>CTTCTCCTCC</u>																							
	<i>5' LIC sequence</i>																												
	<i>BseRI</i>																												

<..... (SacB spacer)  
.....>

**2941** TCGAGGAGTT TACTAGTAAG TAAAGGTGGA TACTCGAGCG GCCGCATCGT GACTGACTGA  
*BseRI* 3' *LIC sequence* *XhoI NotI*

#### Primers for LIC cloning:

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

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

### pGTVL1-SGC sequence:

acatctgttgtcaaagacagcatcattgaacaaggacaattaacagttaacaataaaaacgcaaaaga  
aaatgcgcatacattgtcattggcatttgcactttcgaggagttacttagtaagtaaagg  
ggataactcgagcggccgatcgactgactgacatctgcgcgcgttcggatgcacgggtaaaa  
acctctgacacatgcagctcccgagacggtcacagctgtctgtaaagcggatgccggagcagacaag  
cccgtcagggcgcgtcaggggttgcgggtgtcggggcgcagccatgaccactacgttagcgata  
gcggagtgtataattcttgaagacgaaaggccctcgatgcgcattttataggttaatgtcatga  
taataatggtttttagacgtcaggtggactttcgggaaatgtgcgcgaaccctatttttat  
tttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaaatgcttaataatt  
gaaaaaaggaagagatgagttttcaacattccgtgcgccttattcccttttgcggcattttgc  
ttcctgttttgcaccagaaacgctggtaaaagatgctgaagatcagttgggtgcacgag  
tgggttacatcgaactggatctcaacagcggtaagatccttgagagtttcgccccgaagaacgtttc  
caatgtgagcactttaaagttctgtatgtggcgcgttattatccgtgttgcgcggcaagagc  
aactcgctgcgcgcatacactattctcagaatgacttgggttagtactcaccagtacagaaaaagcatc  
ttacggatggcatgacagactaagagaattatgcagtgcgcataaccatgagtgataacactgcggcca  
acttacttctgacaacgcatacgaggaccgaaggagactaccgcattttgcacaacatggggatcatg  
taactcgccctgtatcggtggaaaccggagctgaatgaagccataccaaacgacgacgcgtgacaccacga  
tgcctgcagcaatggcaacaacgttgcgcaactattacttgcgactactactctagctccggc  
aacaattaatagactggatggaggcgataaaagttgcaggaccacttcgcgcgcgcgcgc  
gctggttattgtgataaaatctggagccggtagcgtgggtctcgccgtatcatgcagactggggc  
cagatgtaagccctccgtatcgtagttatctacacgacggggagtgcaggcaactatggatgaacgaa  
atagacagatcgctgagataggtgcctcactgatcatgcggatctgcagaccatgttactcat  
atatacttagattgattnaaacttcattttattnaaaggatcttaggtgaagatcctttgata  
atctcatgaccaaaatccctaactgtgagtttcgttccactgagcgtcagaccctgttagaaaagatca  
aaggatcttgcgatcccttttcgcgtatctgcgttgcggataacaaaaaccaccgc  
cagcgggtttttgcggatcaagagactaccacttttcgcggatctggccaccactcaagaactctgttagc  
cgcagataccaaactgtcctctgtatccgttaccactgtggctgcccactggcgtatccgtt  
ccgggttgcgactcaagacgatgttaccggataaggcgcagcggtatccgttgcggatctgcgtt  
cacagcccagcttggagcgaacgcacccatcaccgaactcgagatcatgcgttagatgagaaagcg  
ccacgcttccgaagggagaaaggcggacaggatccgttaagcggcagggtcggaacaggagacgc  
cgaggagcttccagggggaaacgcctgtatctttagtgcgttgcggatctgcgtt  
agcgtcgatttgtatgcgtcgtcaggggggcgagctatggaaaaacgcgcacgcgcgc  
tacggttcctggcctttgcgcctttgcgtatccgtatccctgtattctgtgg  
ataaccgtattaccgccttgcgtatgcgttgcgtatccgtatccctgtattctgtgg  
cagtgcgcggaaagcggaaagagcgcctgtatgcgttgcgtatcccttgcgtatccgtatcc  
accgcataaattccgcacccatcgaatgtgcggatccgtatccgtatccgtatccgtatcc  
agatcattcagggtggtaatgtgaaaccaggtaacgttatacgatgtcgcagatgtccgggtgtct  
ttatcagaccgttccgcgtggtaaccaggccacgttctgcgaaaacgcgggaaaaagtgg  
aagcggcgatggcgagactgaattacatcccaaccgcgtggcacaacaactggcggcaacagtcgt  
tgctgatggcgatgcgcacccacttcgtcgtcggccctgcacgcgcgtcgcaatttgcgc  
ctcgccgcgtatcaactgggtgcgcgtggatgcgtatccgtatccctgtatccctgt  
aagcggcggtgcacaatcttcgcgcacgcgtcgtggatccgtatccctgtatcc  
aggatgccattgcgtggaaactgcgtcgtcgttgcgttgcgttgcgttgcgttgcgt  
caccatcaacagtattatccatgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tgggtcaccagcaatcgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gctggcataaatatctactcgtcaatcaaattcgcgttgcgttgcgttgcgttgcgt  
tgtccgtttcaacaaaccatgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
acgtcagatggcgatggcgatgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
cggtagtggatacgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
attttcgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gcaatcagctgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
ctcccccgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gagcgcacaacgcataatgtgatgttgcgttgcgttgcgttgcgttgcgttgcgt  
gctcgatgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
ggattcactggccgtcggttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tgcagcacatcccccgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gctggagatgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gacgggttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt

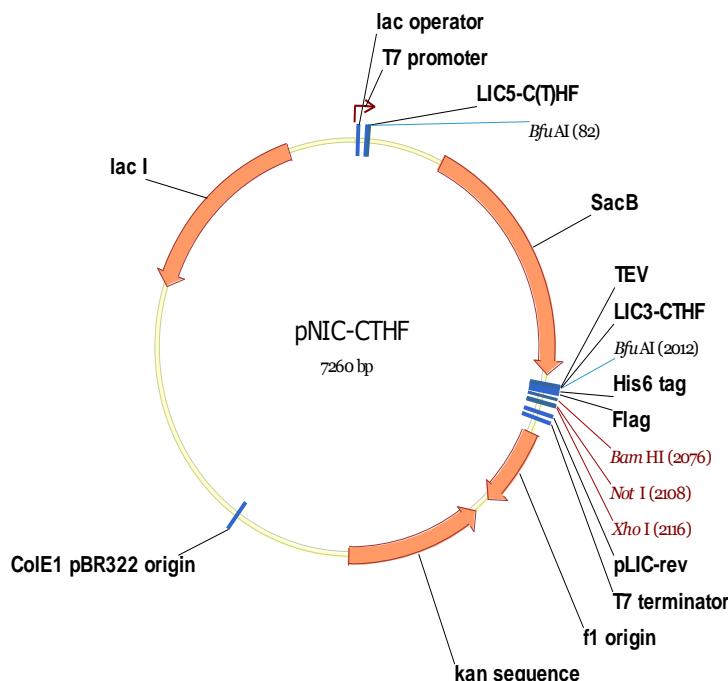
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC-CTHF</b>
Source	Opher Gileadi
Sequence accession/link	EF199844

Description	pET expression vector with C-terminal His <sub>6</sub> tag and FLAG tag, preceded by a TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	AENLYFQ*SHHHHHHDYKDDDDK (* - TEV cleavage site)
C-terminal fusion – MW	2771 (1905 Da removed by TEV cleavage)
Termination codons	supplied in vector
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



Polylinker region:

5' end:

*BfuAI*  
~~~~~  
CTAGAAATAA TTTGTTAA CCTTAAGAAG GAGA|TATA CT AT**GCAGGT**CG TTCACTATTA  
GATCTTATT AAAACAAATT GGAATTCTTC CTCT ATAT|GA TACGTCCAGC AAGTGATAAT

----- SacB fragment -----

*BfuAI*  
~~~~~  
A E N L Y F  
CCGATATCCT ATTGGCATTG ACGTCAGGTG GCAC**ACCTGC** AGAG|AA CC TCTACTTC  
GGCTATAAGGA TAACCGTAAC TGCAGTCCAC CGTGT**GGACG** TCTC TT GG|AGATGAAG  
-----  
(TEV---  
|  
Q S H H H H H H D Y K D D D D K \* BamHI  
1981 CAATCGCACC ATCATCACCA CCATGATTAC AAGGATGACG ACGATAAGTG AGGATCCGAA  
GTTAGCGTGG TAGTAGTGGT GGTACTAATG TTCCTACTGC TGCTATTACAC TCCTAGGCTT  
-----  
-TEV) His6 tag Flag

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.

pNIC-CTHf sequence:

taatacgactcactatagggaattgtgagcggataacaattccctctagaataatttgttaacc  
ttaagaaggagatatactatgcaggctgttactattatttagtgaaatgagatattatgtatttct  
gaatttgtgattaaaaggcaactttatgcccattgcacagaaaactataaaaaatacagagaatgaaaag  
aaacagatagatttttagttctttaggcccgtagtctgc当地atcctttatgatttctatcaaacaa  
aagagggaaaatagaccgttgc当地atccaaacgagagtctaataatgaggtcgaaaagtaaatcgcgc  
gggtttgttactgataaaggcaggcaagcacctaaatgtgtaaaggc当地aaagtgtatactttggcgtcac  
cccttacatatttttaggtcttttattgtgc当地actacttgc当地atcttcaaacaggaggc当地tgg  
agaagcagaccgctaaacacagttacataaaaaaggagacatgaacatcaaaaagttgcaaaa  
caagcaacagtattaaccttactaccgc当地actgtggc当地aggaggc当地actcaagc当地ttgc当地aaaagaa  
acgaaccaaaagc当地atataaggaaacatacggc当地atttcccatattacacgc当地atgctg当地aaaatc  
cctgaacagcaaaaaatgaaaaatataaagttc当地tgc当地atcc当地c当地atcaaaaatatct  
tctgc当地aaaggc当地tgg当地gttgg当地acagtc当地ggc当地attacaaaactgc当地acggc当地actgtgc当地aaaact  
cacggc当地taccacatcgt当地ttgc当地tagcc当地ggagatc当地taaaaatgc当地ggatgc当地acatc  
ttctatcaaaaatgtc当地ggc当地aaacttctattgc当地agc当地gtgg当地aaaacgc当地tggc当地gtctt当地aaagacagc  
gacaaaattc当地gtg当地aaatgattctatc当地taaaaagaccaacacaagaatggt当地cagggtc当地aggccat  
acatctgacggaaaatccg当地ttattctacactgattctcc当地gttaaaaacattacggc当地aaacacaact  
acaactgc当地acaaggtaacgtatc当地gcatc当地gacacaactgc当地tcttgc当地aaacatcaac  
tcaatcttgc当地cggc当地acggaaaacgtatc当地aaatgtacagc当地cagttc当地atc当地gat  
gaaggc当地actacagc



ggccgcgcataatggcctgcttcggaaacgttggggaccagtgcgcaggc  
ttgagcgaggcgatcgaaataccgcacaggccatcatcgccgcctccaggc  
gcggctcgccgaaaatgacccagagcgctggccacctgtcctacgaggatgc  
atcataatgcggcgacgatgtcatgccccgcgcccaccggaggactgactgg  
caaggcatcggtcgagatcccggcctaattgactgatcgatcattacattat  
gcccgcggaaacctgtcgccagtcgatcgatcgatcgatcgatcgatcg  
cggttgcgtattggcgccagggtgtttctttcaccagtgcggacacag  
tcaccgcctggccctgagagatgtcgagcaagcggtccacgctgg  
gtttgatgggttaacggggatataacatgatcgatcgatcgatcgatcg  
tatccgcaccaacgcgcagccggactcgtaatggcgccatcgccatct  
caaccagcatcgcgatggAACGATGCCCTATTAGCAGCATGGTGT  
cactccagtcgcctccgtatcggtgaattgattgcgagtgagatattat  
ccagacgcagacgcgcggagacagaacttaatggccgccta  
cgaccagatgctccacgcggactcgatcgatcgatcgatcgatcg  
ggtagagacatcaagaataacgcggaaacattagtcaggc  
catccagcggatagttaatgatcgccactgacgcgttgc  
aggcttcgacgcgcgttgcgttaccatcgacaccac  
taatcgccgcacaatttgcacggcgctgcagg  
actgttgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
cttttccgcgtttcgacggatgtggctggctgg  
caccggcatacttcgcacatcgatcgatcgatcgatcgatcgatcg  
ccggcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
cccttatgcgcactcgcattaggaaggc  
aggatggtgcatgcaggatggcccaac  
ccgaaacaagcgctcatgaggccgaatggcg  
gcgcgcaggcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
tcgatccgcgaaat

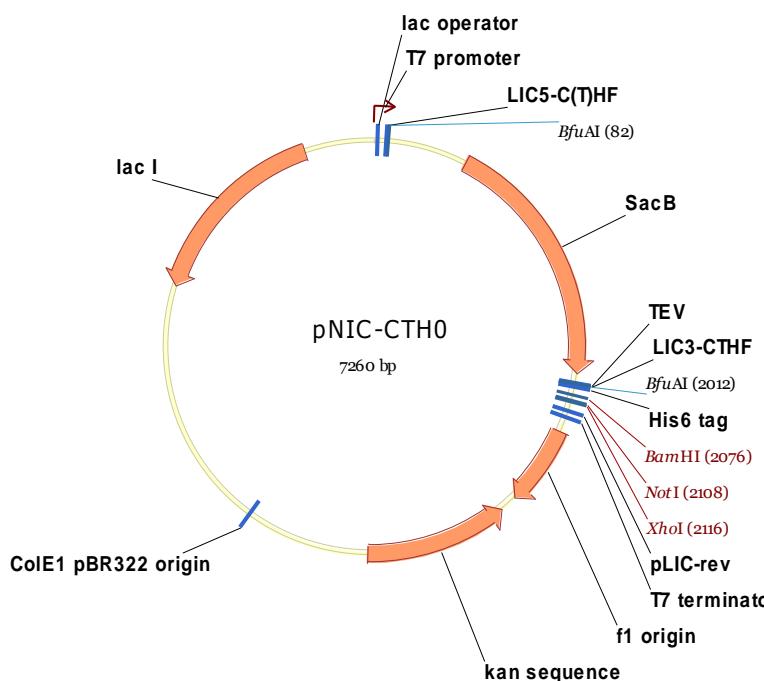
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC-CTHO</b>
Source	Pavel Savitsky
Sequence accession/link	

Description	pET expression vector with C-terminal His <sub>6</sub> tag, preceded by a TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)
Initiation codon	Supplied in PCR primer
C-terminal fusion – seq.	AENLYFQ*SHHHHHHH (* - TEV cleavage site)
C-terminal fusion – MW	1793 (1077 Da removed by TEV cleavage)
Termination codons	supplied in vector
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



Polylinker region:

5' end:

*BfuAI*  
~~~~~

CTAGAAATAA TTTTGTAA CCTTAAGAAG GAGA|TATA CT AT**GCAGGT**CG TTCACTATTA  
GATCTTATT AAAACAAATT GGAATTCTTC CTCT ATAT|GA TACGTCCAGC AAGTGATAAT

----- SacB fragment -----

*LIC3-CTHF*  
~~~~~

TEV His6 tag  
~~~~~ ~~~~~

*BfuAI*  
~~~~~

T P A E N L Y F Q S H H H H .

1981 GGCATTGACG TCAGGTGGCA CACCTGCAGA GAACCTCTAC TTCCAATCGC ACCATCATCA  
CCGTAACCTGC AGTCCACCCT GTGGACGTCT CTTGGAGATG AAGGTTAGCG TGGTAGTAGT  
His6 tag  
~~~~~

*BamHI*  
~~~~~

· H H

2041 CCACCATTGA TACAAGGATG ACGACGATAA GTGAGGATCC GAATTCGAGC TCCGTCGACA  
GGTGGTAACT ATGTTCCCTAC TGCTGCTATT CACTCCTAGG CTTAAGCTCG AGGCAGCTGT  
NotI XhoI  
~~~~~ ~~~~~

2101 AGCTTGCAGC CGCACACTCGAG CACCACCAACC ACCACCCTG AGATCCGGCT GCTAACAAAG  
TCGAACGCCG GCGTGAGCTC GTGGTGGTGG TGGTGGTGAC TCTAGGCCGA CGATTGTTTC

2161 CCCGAAAGGA AGCTGAGTTG GCTGCTGCCA CCGCTGAGCA ATAACTAGCA TAACCCCTTG  
GGGCTTCCT TCGACTCAAC CGACGACGGT GGCGACTCGT TATTGATCGT ATTGGGGAAC  
~~~~~

pLIC-rev

2221 GGGCCTCTAA ACGGGTCTTG AGGGGTTTT TGCTGAAAGG AGGAACATATA TCCGGATTGG  
CCCGGAGATT TGCCCCAGAAC TCCCCAAAAA ACGACTTCC TCCTTGATAT AGGCCTAAC

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATACTATATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.

pNIC-CTHO sequence:

taatacgactcaactatagggaattgtgagcggataacaattccctctagaataattttgttaacc  
ttaagaaggagatatacatatgcaggctgttactattatgtaaatgagatattatgtatatttct  
gaatttgtgattaaaaaggcaactttatgcccattgcacacagaaaactataaaaaatacagagaatgaaaag  
aaacagatagatttttagtctttaggcccgtagtctgcataatcctttatgatttctatcaaacaa  
aagagaaaaatagaccgttgcataatccaaacgagagtctaataatagaatgaggtcgaaaagtaaatcgcc  
gggtttgttactgataaaggcaggcaagacctaataatgtgtaaaggcataatgttgcgtcac

cccttacatatttagtcttttattgtcgtaactaacttgccatcttcaaaccaggagggctgga  
agaagcagaccgctaacaacagactacataaaaaaggagacatgaacatcaaaaagttgcaaaa  
caagcaacagtattaaccttactaccgcactgtggcaggaggcgcaactcaagcgttgcgaaagaa  
acgaaccaaaagccatataaggaaacatacggcattcccatattacacgcccattacatgcgatatgct  
cctgaacacagcaaaaaatgaaaaatataaaagttcctgagttcgattcgccacaattaaaaatatct  
tctgcaaaaaggcctggacggtggacagctggcattacaaaacactgacggcactgtgcacaactat  
cacggctaccacatcgtcttgattagccggagatcctaaaaatgcggatgacacatcgattacatg  
ttctatcaaaaagtcggcggaaacttctattgacagctggaaaaacgctggccgcgtcttaagacagc  
gacaaattcgatgcaaatgattctatctaaaagaccaaaacacaagaatggtcaggttagccacat  
acatctgacggaaaaatccgttatttacactgattctccggtaaacattacggcaaaacactg  
acaactgcacaaggtaacgtatcagcatcagacagctttgaacatcaacggtagaggattaaa  
tcaatcttgcggtgacggaaaaacgtatcaaattgtacacgacgttgcattacatgcgatgaaagg  
tcaggcggacaaccatacgctgagagatcctactacgtagaagataaaggccacaatacttagtatt  
gaagcaaacactggaactgaagatggctaccaaggcgaagaatctttatthaacaaagcatactatggc  
aaaagcacaatcattttccgtcaagaaagtcaaaaacttctgcaagcgataaaaaacgcacggctgag  
ttagcaaacggcgcttcggatgattgagctaaacgatgattacacactgaaaaaaagtgtatgaaaccg  
ctgattgcataacacagataacagatgaaattgacgcgcgaacgtctttaaatgacggcaatgg  
tacctgttcaactgactcccgatcaaaaatgacgattgacggcattacgtctaacgatatttacatg  
cttggtatgtttctaatttttaactggccatacaagccgctgaacaaaactggcctgtttaaaa  
atggatcttgcataacgatgtaaccttacttactcacacttcgctgtacctaagcgaaaggaaac  
aatgtcgtgattacaagctatgacaaaacagaggattctacgcagacaaacaatcaacggtgcgcct  
agttcctgctgaaacatcaaaaggcaagaaaacatctgttgcataagacagcatcctgaaacaaggacaa  
ttaacagttacaaaataaaaacgcaaaaggaaaatgccgatattttgcattgacgtcaggtggcac  
acctgcagagaacctctactccaatcgcaccatcatcaccaccattgatacaaggatgacgacgataa  
gtgaggatccgaaattcgagctccgtcgcacaagctgccccactcgagcaccaccaccacc  
gagatccggctgctaacaaaggccgaaaggaaagctgagttggctgtccaccgctgagcaataactag  
cataacccttgggcctctaaacgggcttgcagggtttttgtgaaaggaggaactatattccggat  
tggcgaatggacgcgcctgttagcggcgcattaagcgcggcggtgtgggttacgcgcacgcgtgac  
cgctacacttgcacgcgccttagcgcgcgccttcgcctttttcccttcgcacgcgttgc  
cggtttcccgtaagctotaatgggggtcccttagggttccgatttagtgcattacggcac  
cgaccaaaaacttgattagggtatggtcacgttagtgcgcattgcgcgcataagcgttt  
cccttgcgttgcggacttcaatagtgactctgttccaaactggaacaacactcaaccc  
tatctcggtctattttgattataaggatttgccgatattcgccatttgcgttgcatttt  
gattnaacaaaaatttaacgcgatatttacaaaatattacgatgcgttgcacttt  
ggaaatgtgcggaaaccctatttttattttcaatattacattcaaatatgtatccgctcatgaa  
ttaatttttagaaaaactcatcgagcatcaatgaaactgcaatttattcatatcaggattcaata  
catattttgaaaaaggcgttctgtaatgaaggagaaaactcaccgaggcagttccataggatggcaa  
gatcctgttatcggtctgcgattccgactcgatccaaacatcaataacacttatttccctcgtaa  
aaataaggatcaagtgagaaatcaccatgagtgcgactgaatccggtgagaatggcaaagtttat  
gcattttcccgacttgtcaacaggccaggccattacgcgtcatcaaatactcgcatcaacca  
aaccgttattcattcggtattgcgcctgagcgagacgaaatacgcgatcgctgtt  
aaacaggaatcgaaatgcacccgcgcaggaaacactgcccgcgcataacaatatttccat  
gatattttcaatactggatgctgtttccgggatcgacgtggtagtaaccatgcacatcat  
gagtagcgataaaatgcttgcgttgcggaaagaggcataaattccgtcgcgcattt  
catctgttaacatcatggcaacgcgttgcgcatttgcgcattatcgacgcgcattt  
catacatcgatattgtgcgcatttgcgcattatcgacgcgcattt  
cagcatccatgttgcatttgcgcattatcgacgcgcattt  
ccctgttattactgtttatgttaagcagacgattttatttgcgcattt  
tcgttgcactgagcgctgagcccgtagaaaagatcaaggatcttgcgcattt  
gtaatctgctgtcaacaaaaaccaccgcgttgcgcattt  
ccaacttttccgaaggtaactggcttgcgcatttgcgcattt  
ccgttagttaggcaccacttcaagaactctgttagcaccgcct  
ccagtggctgctgccagtgccgataagtgcgttaccgggt  
aaggcgcagcgctggctgaacgggggttcgtgcacac  
gaacttagagataccatcagcgtagctatgagaa  
tatccggtaagggcagggtcggaaacaggag  
ctttagtgcggatcagg  
cgagctatggaaaaacgc  
cacatgttctgcgtt  
accgctcgccgc  
cggtattttctc  
cgatctgt  
cgatc  
actctc  
actat



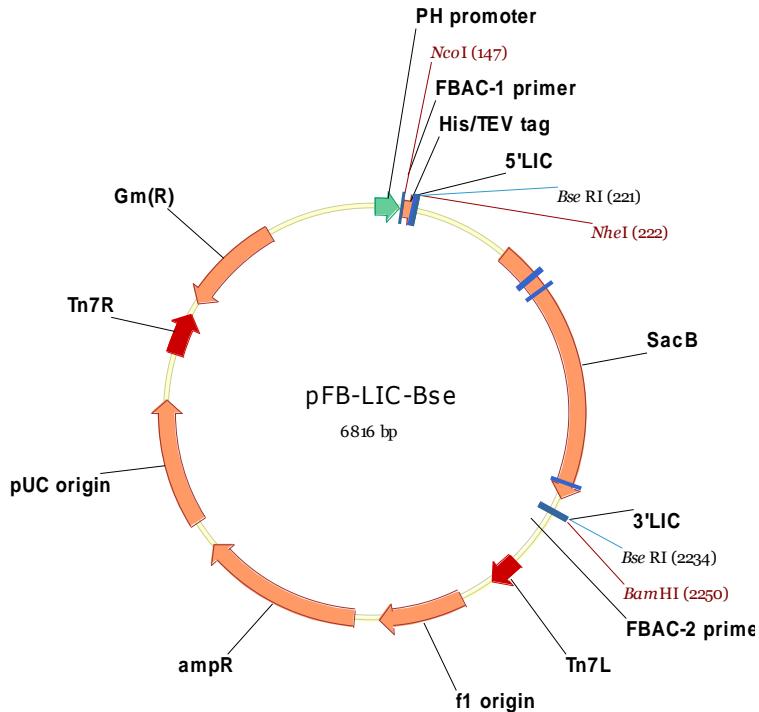
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pFB-LIC-Bse</b>
Source	Opher Gileadi
Sequence accession/link	EF199842

Description	Baculovirus transfer vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site. Includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection of transformed bacteria on 5% sucrose
-------------	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Ampicillin, 100 µg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2630 Da including Met (2411.8 Da removed by TEV cleavage)
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA
5' sequencing primer	FBAC1: TATTCATACCGTCCCACCA
3' sequencing primer	FBAC2: GGGAGGTTTTAAAGCAAGTAAA



### Polylinker region:

FBAC-1 primer  
~~~~~  
61 TTATTCACTAC CGTCCCCACCA  
AATAAGTATG GCAGGGTGGT

NcoI  
~~~~~  
121 M G H H H H H H H S S G  
TCGGGC CGCG ATCTCGGTCC GAAAACCATG GGCCACCATC ATCATCATCA TTCTTCTGGT  
AGCCCGCGCC TAGAGCCAGG CTTTTGGTAC CCGGTGGTAG TAGTAGTAGT AAGAAGACCA

BglII Lic5' BseRI  
~~~~~ ~~~~~ ~~~~~  
V D L G T E N L Y F Q S  
181 GTAGATCTGG GTACCGAGAA CCTG**TACTTC** CAATCCATAA GCTAGCTTCT CCTCCTGAAA  
CATCTAGACC CATGGCTCTT GGAC**ATGAAG** GTTAGGTATT CGATCGAAGA GGAGGACTTT

--SacB linker--  
2161 BseRI  
~~~  
ACTTTTCGAG  
TGAAAAGCTC

BamHI  
~~~~~  
2221 BseRI Lic3'  
~~~ ~~~~~ ~~~~~  
GAGTTTACTA GTA**AGTAAAG** GTGGATA**CGG** ATCCGAATTC GAGCTCCGTC GACAAGCTTG  
CTCAAATGAT CAT**TCATTTC** CACCTAT**GCC** TAGGCTTAAG CTCGAGGCAG CTGTTCGAAC

### Primers for LIC cloning:

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

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

pFB-LIC-Bse sequence:

aatgaaggccataccaaacgacgacgctgacaccacgatgcctgttagcaatggcaacaacgttgcgcaaa  
ctattaactggcgaactacttactctagcttcccgcaacaattaatagactggatggaggcggataaa  
gttcaggaccacttctgcgctcgcccttccggctggctggttattgctgataaatctggagccggt  
gagcgtgggtctcgcttatattgcagcactggggccagatgtaagccctccgtatctgttagttatc  
tacacgacggggagtcaggcaactatggatgaacaaaatagacagatcgctgagataggtgcctcactg  
attaagcatggtaactgtcagaccaagttactcatatatacttttagattgattaaaacttcatttt  
taattttaaaaggatctaggtgaagatcctttgataatctcatgaccaaattccctaacgtgagtt  
tcgttcactgagcgtcagacccgtagaaaagatcaaaggatcttcttgagatccttttctgcgc  
gtaatctgctgttgcacaaaaaccaccgctaccagcgggtggttgcggatcaagagact  
ccaacttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtcctctagttag  
ccgttagttaggccaccacttaagaactctgttagcaccgcctacatacccgctctgtaatccgtta  
ccagtgcgtgcgcagttgcgataagtcgttaccgggtggactcaagacgatagttaccggat  
aaggcgcagcggctggctgaacgggggttcgtcacacagcccagctggagcgaacgaccc  
gaactgagataacctacagcgtgagcattgagaaagcgcacgcctccgaagggagaaaggcggacagg  
tatccgttaagcgcagggtcggaacaggagagcgcacgcaggagcttccaggggaaacgcgttgtat  
ctttatagtccgtcggttgcgcacccctgacttgcgtgatttgtatgcgtcagggggg  
cgagccatggaaaaacgcgcagcaacgcgcctttacgcgttgcgcctttgcgcctttgc  
cacatgttcttcctgcgttatccctgattctgtggataaccgtattaccgccttgagtgc  
accgcgcgcgcagccgaacgaccgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
cggtatcccttcctgcgttatccctgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ggttacgggttagtaataatggatgcctgcgttaagcgggtgtggcggacaataaagtcttaaactg  
aacaatagatctaaactatgacaataaagtcttaaactagacagaatagttgttaaactgaaatc  
ccagttatgcgtgaaaaagcataactggactttgttatggctaaagcaaactcttcatttgc  
gcaaatggccgcgttataaagaggggcgtggccaaggcatgtaaagactatattcgcggcgttgc  
gacaattaccgaacaactccgcggccggaaagccgatctggctgaacgaaattgttaggtggc  
cttgggtcgatataaagtgcactacttctccgtatgccactttgtatagagagccactgc  
tcgtaccgtatgcgtcgttagatcacataagcacaaggcgcgttgcgcgcgcgcgc  
ttgatgagcgcggcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
tcactacgcggcgtctcaaacctggcagaacgtaagccgcgcgcgcgcgc  
aaggcagcaagcgcgtatgtcttactacggagcagaattcccgaggtatcg  
tgggagtaggtggctacgtctccgaactcagcaccggaaaagatcaagagc  
tggcggccgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
ctggccctgcgtgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
cagccgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
catgaaaaccgcactgcgcgttaccaccgcgtgcgttgcgttgcgttgcgttgcgttgcgttgc  
catacgctacttgcattacagttacgaaccgaacaggcttgcgttgcgttgcgttgcgttgc  
tttccacgggtgtgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
gaacgagcgaagggttcggctccacgcgtcaggcattggcgcgcgcgc  
ggtgcgtgcacggatctggccctggcttgcgttgcgttgcgttgcgttgcgttgc  
ggtgcgtaccccgatgaagtgggttgcgttgcgttgcgttgcgttgcgttgc  
ggactctagctatagttctagttgcgttgcgttgcgttgcgttgcgttgcgttgc

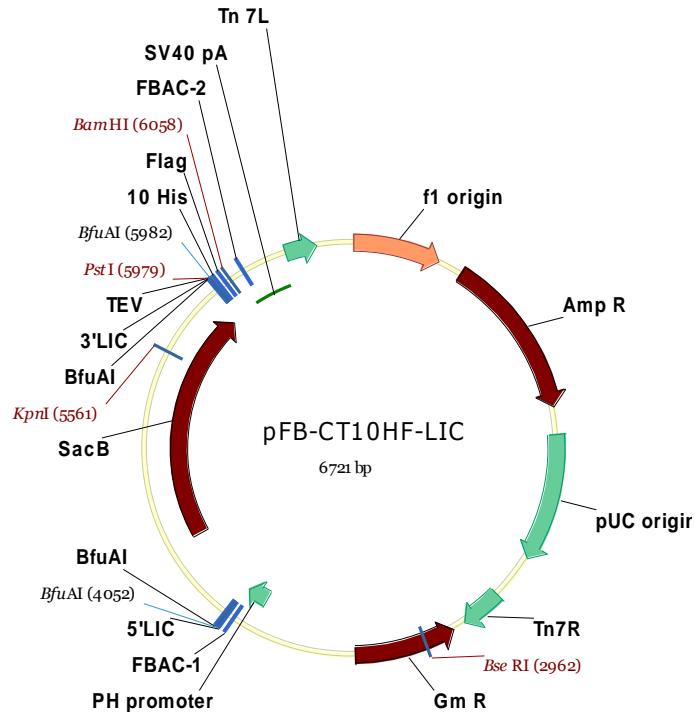
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

|                         |                       |
|-------------------------|-----------------------|
| Vector Name             | <b>pFB-CT10HF-LIC</b> |
| Source                  | Grazyna Kochan        |
| Sequence accession/link | (SGC)                 |

|             |                                                                                                                                                                                                                                                           |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Description | Baculovirus transfer vector with C-terminal His <sub>10</sub> tag and FLAG tag, preceded by a TEV protease cleavage site. 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                 | Polyhedrin                                                                                                                      |
| Cloning                  | LIC. (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)                                               |
| Initiation codon         | Supplied in PCR primer                                                                                                          |
| C-terminal fusion – seq. | AENLYFQ*SHHHHHHHHHDYKDDDDK<br>(* - TEV cleavage site)                                                                           |
| C-terminal fusion – MW   |                                                                                                                                 |
| Termination codon        | Downstream of flag tag                                                                                                          |
| Protease cleavage        | TEV                                                                                                                             |
| Additional features      | Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).                      |
| Preferred host           | Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA |
| 5' sequencing primer     | FBAC1: TATTCATACCGTCCCACCA or<br>FBAC3: TTAAAATGATAACCATCTG                                                                     |
| 3' sequencing primer     | FBAC2: GGGAGGTTTTAAAGCAAGTAAA                                                                                                   |



### Polylinker region:

5' end:

BfuAI  
~~~~~  
4021 CCATCGGGCG CGGATCTCCT TAAGAAGGAG ATATACTATG CAGGTGGTTC ACTATTATTT  
GGTAGCCCGC GCCTAGAGGA ATTCTTCCTC TATATGATAC GTCCAGCAAG TGATAATAAA

### SacB fragment

TEV  
~~~~~  
3'LIC  
~~~~~  
BfuAI 10 His  
~~~~~  
BfuAI ~  
~~~~~  
PstI  
~~~~~  
A E N L Y F Q S H .  
5941 ATATCCTATT GGCATTGACG TCAGGTGGCA CACCTGCAGA GAACCTCTAC TTCCAATCGC  
TATAGGATAA CCGTAACTGC AGTCCACCGT GTGGACGTCT CTTGGAGATG AAGGTTAGCG  
10 His  
~~~~~  
Flag  
~~~~~  
BamHI  
~~~~~  
· H H H H H H D Y K D D D D K  
6001 ACCATCATCA CCATCACCAT CACCACCATG ATTACAAGGA TGACGACGAT AAGTGAGGAT  
TGGTAGTAGT GGTAGTGGTA GTGGTGGTAC TAATGTTCCCT ACTGCTGCTA TTCACCTCCTA

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATATATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.

pFB-CT10HF-LIC sequence:

gacgcgccctgttagcggcgattaaagcgcggcggtgtggtggttacgcgcagcgtgaccgctacactt  
gccagcgcctagcgcgcgtcccccgtttcgcttctcccttcgttcgcacgttcgcggcttcccc  
cgtcaagctctaaatcgggggccgttttagggttcgatttagtgccttacggcacctcgacccaaa  
aaacttgattagggtgatggttacgttagtggccatgcgcgtatagacggttttcgcgccttgcg  
ttggagtcacgtttaatagtggactcttgccttcaacttgcgatagacttgcggaaatgt  
tattctttgattataaggatgttgcgcatttgcgcatttgcgttgcggaaatgt  
aaatattaacgcgaatttacaacaaaatattaacgttacaatttcaggtggcactttcgggaaatgt  
cgccgaacccctattgttatttctaaatacatcaaatatgtatccgctcatgagacaataaccc  
tgataaatgttcaataatattgaaaaaggaagagtatgagtttgcgttgcgccttatt  
ccctttttgcgcatttgcctctgtttgcgcatttgcgcatttgcgttgcggaaatgt  
gaagatcagttgggtgcacgactgggttacatgcgcacttgcgcatttgcgttgcggaaatgt  
tttcgcggcgaagaacgtttcaatgtatgagactttttgcgttgcgttgcggattatcc  
cgtattgcgcggcaagagcaactcggtgcgcgcataacttgcgttgcgcatttgcgttgcgc  
tcaccagtacagaaaaagcatcttgcgttgcgcatttgcgttgcgcatttgcgttgcgc  
atgagtgataacactgcggccaacttacttgcgttgcgcatttgcgttgcgcatttgcgttgcgc  
ttgcacaacatggggatcatgttacttgcgttgcgcatttgcgttgcgcatttgcgttgcgc  
aacgacgacgcgtgacaccacgcgttgcgttgcgcatttgcgttgcgcatttgcgttgcgc  
ctacttactctagttccggcaacaattaatagacttgcgttgcgcatttgcgttgcgc  
ctgc  
tgtatcattgc  
caggcaactatggatgaacgcggatgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
ctgtcagaccaagttactcatatatacttttagattgatttgcgttgcgcgcgcgc  
taggtgaagatcctttgataatctcatgacaaaaatccctaacgttagtttgcgttgcgc  
tcagacccgtagaaaagatcaaaggatcttgcgttgcgcgcgcgcgcgcgc  
caaacaaaaaccaccgcgttgcgcgcgcgcgcgcgcgcgcgcgcgc  
aaggtaactggctcagcagacgcgcgcgcgcgcgcgcgcgcgc  
cacttcaagaactctgttagcaccgcctacatacctcgctgttgcgcgcgc  
agtggcgataagtgcgttgcgcgcgcgcgcgcgcgcgcgc  
ggctgaacgggggggttcgttgcgcgcgcgcgcgcgcgc  
cagcgttgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
agggtcggacaggagagacgcgcgcgcgcgcgcgcgc  
gggtttcgccacctctgacttgcgttgcgcgcgcgcgc  
aacgcgcacgcggcccttttacgggttgcgcgcgcgc  
gcgttatcccgtatttgcgttgcgcgcgcgcgc  
cgaacgcacgcgcgcgcgcgcgcgcgcgc  
acgcacgcgcgcgcgcgcgcgcgcgc  
ataaatggatgcctgcgttgcgcgcgcgcgc  
aactatgacaataaagtcttgcgttgcgcgcgc  
aaaagcataactggactttttgttatggctaaagcaacttgc  
tattaaagagggcggtggccaaaggcatggtaaagactatatt  
aactccgcggccggaaagccgatctcggttgcgcgc  
aagtgcacgcgcgcgcgcgcgcgcgc  
gcttgcacgttagatcacataagccaccaagcgcgc  
gcaatgcgcgcgcgcgcgcgcgc  
tcaaacctggcagaacgttgcgcgcgc  
atgaatgtcttactacggagcaagttcccgaggt  
atcggagttccgcgtatgttggagtaggtggct

acgtctccgaactcacgaccgaaaagatcaagagcagccccatggatttgacttggtcagggccgagc  
ctacatgtgcgaatgatgccatacttgagccacctaaacttttttagggcactgcccgtgcgt  
acatcggtctgcgtgcgtacatcggtctgcgtccataacatcaaacatcgacccacggcgtaacgcgc  
ttgctgtggatgcccaggcatagactgtacaaaaaaaaaacagtataacaagccatgaaaaccgcac  
tgcgcgttaccaccgtcggtcaagggtctggaccagttgcgtgagcgcatacgtacttgca  
ttacagttacgaaccgaacaggctatgtcaactgggtctgcctcatccgttccacgggtgtgc  
tcacccggcaacctggcagcagcgaactcgaggcattctgtccctggctggcgaacgagcgaaggt  
ttcggctccacgcacgtcaggcattggcggcctgtgttctacggcaaggtgtgcacgg  
tctgcctggcttcaggagatcggaaagacctggccgtcgccgcctgcccgtggcgtgacccgg  
tgaagtgggtcgcatcctcggtttctggaaaggcagcatcggttgcgtcccccaggactctagctata  
ttcttagtgggtggctacgtatactccgaatattaatagatcatggagataattaaatgataaccatc  
tcgcaaaataataagtatTTTactgtttcgtaacagttgtataaaaaaaacctataatattccgg  
attattcataccgtcccaccatggcggatctcctaagaaggagataactatgcaggtcggtca  
ctattatTTtagtggaaatgagatattatgatatttctgaattgtgatttttttaggcaactttatgccc  
tgcaacagaaaactataaaaaatacagagaatgaaaagaaaacagatagatttttagttctttaggccc  
tagtctgcaaatcctttatgatTTTctatcaaacaaaagggaaaatagaccagtgcattccaaacg  
agagtctaataagaatgaggtcgaaaagtaatcgcgcgggttgcgttactgataaagcaggcaagaccta  
aaatgttaaaggcggaaagtgtatactttggcgtcacccttacatatttaggttttttattgt  
cgtaactaacttggcatcttcaaacaggaggctggaaagaaggcagaccgcttaacacagttacataaaaa  
ggagacatgaacgatgaacatcaaaaagttggaaacaaggcaacagtattaacctttactaccgcact  
gctggcaggaggcgaactcaagcgttgcggaaagaaaacgaacccatataaggaaacacatacg  
cattcccatattacacgcattatgtgcggaaatccctgaacagcaaaaaatgaaaatataaagt  
tcctgagttcgattcgccacaattaaaaatatcttctgaaaaggcgtggacggtttggacagctg  
gccattacaaaacactgacggcactgtcgaaactatcaggctaccacatcgctttgcattagccgg  
agatcctaaaaatgcggatgacacatcgatttacatgttctatcaaaaagtcggcggaaacttctattga  
cagctggaaaaacgctggcgcgtctttaagacagcgcacaattcgatgcggaaatgtattctatcctaaa  
agaccaacacaagaatggtcaggttcagccacatttacatctgcggaaaatccgttattctacac  
tgatttccggtaaacattacggcaacaaaacactgacaactgcacaagttacgtatcagcatcaga  
cagctttgaacatcaacgggttagaggattaaaatcaatcttgcgggtacggaaaacgtatca  
aaatgtacagcagttcatcgatgaaggcaactacagctcaggcgcacaaccatacgtgagagatcctca  
ctacgttagaagataaaggccaaaatacttagtatttgcggaaaacactggactgaagatggctacca  
aggcgaagaatcttatttacaaaagcatactatggcaaaaggcacaatcattttccgtcaagaaagtca  
aaaacttgcggaaacgcataaaaaacgtacggctggatggatggatccttgcggatgttgc  
aaacgtgattacacactgaaaaaagtgtatgaaaaccgctgttgcattacacacagtaacagatgaaat  
tgcggcggcaacgtctttaaaatgaaaacggcaatggtacctgttactgcactcccgccgataaaaat  
gacgatttgcggcattacgtctaacgatatttacatgttgcgttgcgttgcgttgcgttgcgttgc  
atacaagccgtgaaaactggccttgtgtttaaaatggatcttgcattacacatgttgcgttgcgttgc  
ttactcacacttcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
aggatttgcggcagacaaaacaatcaacgtttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
atctgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
atgcgtatccattggcatttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
atcatcaccatcaccatcaccatcaccatcaccatcaccatcaccatcaccatcaccatcaccatc  
tccgtcgacaagcttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
cttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
aacttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ttttttactgcattcttagttgtgggttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
tcactgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
aatttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ttaaaaactccattccaccctcccagggtccactatTTTgtccgcccacagcggggcatttttctt  
cctgttatTTTtaatcaacatcctgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
tctgtcacaatgaaaattttctgtcatcttgcgttataatgtttgtatttgcgttgcgttgcgttgc  
gcttatttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc

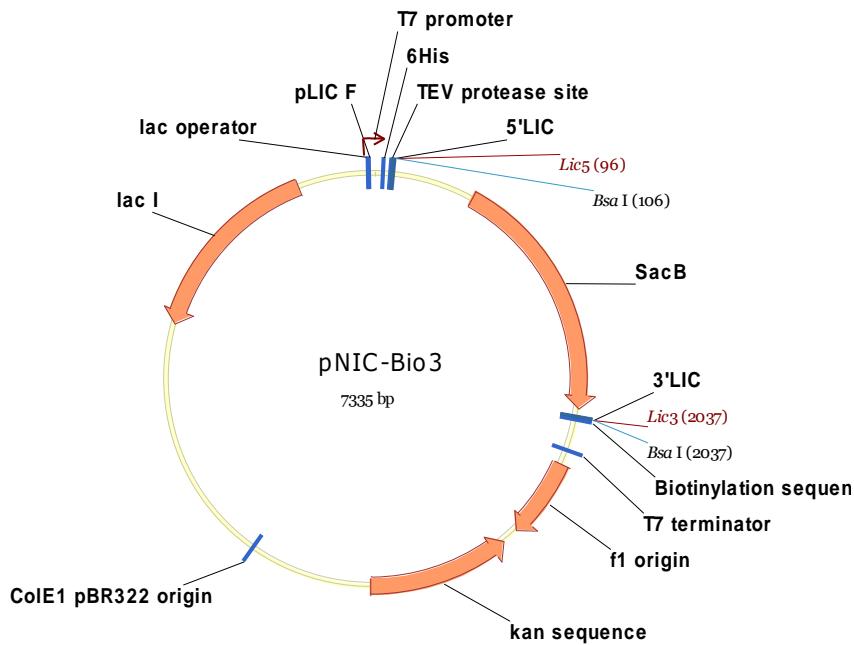
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC-Bio3</b>
Source	Claire Strain-Damerell (derivative of pNIC-Bio2)
Sequence accession/link	JN792439

Description	pET expression vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site, and a biotinylation sequence in C-terminal fusion. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	---

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2684.1 Da including Met (2465.8 Da removed by TEV cleavage)
C-terminal fusion – seq.	GSKGGYGLNDIFEAQKIEWHE
C-terminal fusion - MW	2396.57 Da
Termination codons	Already included after biotinylation signal at C-terminal
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase. For effective biotinylation, the host should overexpress BirA (e.g. from plasmid pCDF-BiRA, Genbank: JF914075)
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



Polylinker region:

M H H H H H H .

1 CTAGAAATAA TTTGTTAA CTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC  
GATCTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG

Lic5 BsaI  
~~~~~ ~~~~~

61 ATTCTCTG GTAGATCTG GGTACCGAGA ACCTGTACTT CCAATCCATG GAGACCGACG  
TAAGAACGACC ACATCTAGAC CCATGGCTCT TGGACATGAA GGTTAGGTAC CTCTGGCTGC

-----SacB-----

BsaI Lic3  
~~~~~ ~~~~  
S K .

1981 TAACAAATAA AAACGCAAAA GAAAATGCCG ATATCCTATT GGCATTGACG GTCTCCAGTA  
ATTGTTATT TTTGCGTTT CTTTACGGC TATAGGATAA CCGTAACTGC CAGAGGTCAT

Lic3  
~~~~~

2041 AAGTGGATA CGGCCGAAT GATATCTTG AAGCGCAGAA GATTGAATGG CATGAATGAT  
TTCCACCTAT GCCGGACTTA CTATAGAAAC TTTCGCGTCTT CTAACCTTACG GTACTTACG

### Primers for LIC cloning:

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

Downstream: add TATCCACCTTACTGCT to 5' end of downstream primer.

### pNIC-Bio3 sequence:

ctagaataatttgtttaacttaagaaggagatatacatatgcaccatcatcatcatcattttctg  
gttgtatctggataccggaaacctgtacttccatccatggagaccggacgtcccacatataacctggcgat





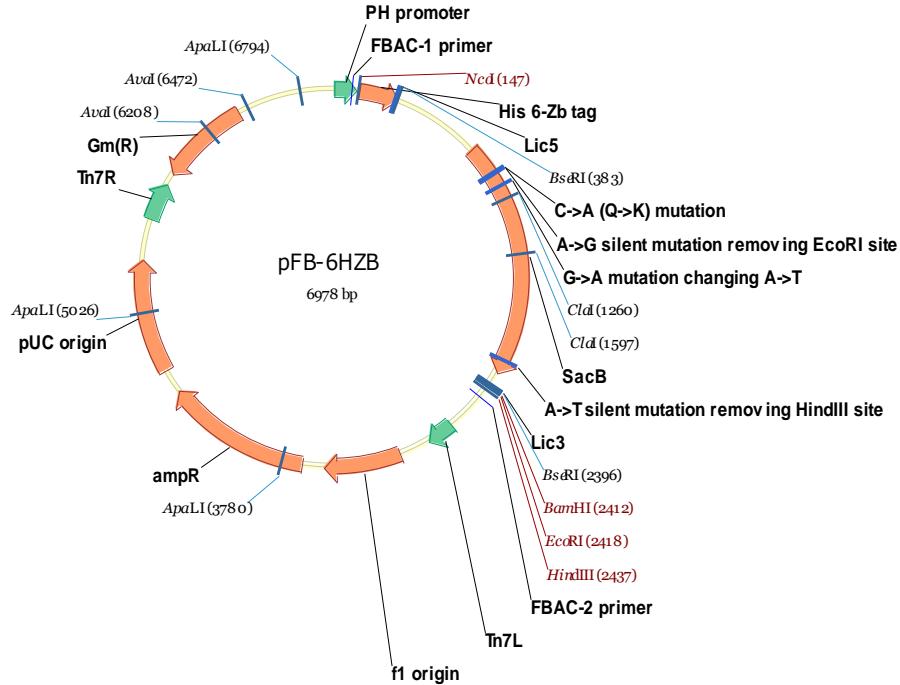
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pFB-6HZB</b>
Source	Pravin Mahajan
Sequence accession/link	

Description	Baculovirus transfer vector with His <sub>6</sub> and Z-basic tags, followed by a TEV protease cleavage site. The Z-basic tag ( <i>J. Chromatog A</i> , 1161:22-28) is a 54-aa sequence derived from protein A and modified to have a high positive surface charge, allowing the fusion proteins to bind to S-sepharose at salt concentrations in which most cellular proteins do not bind. Both the His6 tag and the Zb tag allow purification at stringent conditions. The vector includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection of transformed bacteria on 5% sucrose
-------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Ampicillin, 100 µg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLR DDPSQSANLLAEAKKLNDAQPKGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	9119 Da including Met
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA
5' sequencing primer	FBAC1: TATTCATACCGTCCCACCA
3' sequencing primer	FBAC2: GGGAGGTTTTAAAGCAAGTAAA



#### Polylinker region:

NcoI  
~~~~~

|     |   |                       |
|-----|---|-----------------------|
| 121 | TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCCACCATC ATCATCATCA TTCTTCTGGT   | M G H H H H H H S S G |
|     | AGCCCGGCC TAGAGCCAGG CTTTGGTAC CCGGTGGTAG TAGTAGTAGT AAGAAAGACCA    |                       |
|     | V D N K F N K E R R R A R R E I R H L P                             |                       |
| 181 | GTGGATAACA AGTTAACAA GGAGCGTCGA AGAGCTGCC GTGAAATTG CGCATCTGCCG     |                       |
|     | CACCTATTGT TCAAGTTGTT CCTCGCAGCT TCTCGAGCGG CACTTTAACG GGTAGACGGC   |                       |
|     | N L N R E Q R R A F I R S L R D D P S Q                             |                       |
| 241 | AACCTGAACC GCGAACAGCG TCGCGCATT ATTTCGAGCC TGCGCGATGA TCCGAGCCAG    |                       |
|     | TTGGACTTGG CGCTTGTGCG AGCGCGTAA TAAGCGTCGG ACGCCTACT AGGCTCGTC      |                       |
|     | S A N L L A E A K K L N D A Q P K G T E                             |                       |
| 301 | AGCGCGAACCC TGCTGGCGGA AGCGAACAGAG CTGAACGATG CGCAGCCGAA GGGTACAGAG |                       |
|     | TCGCGCTTGG ACGACCGCCT TCGCTTCTTC GACTGCTAC GCGTCGGCTT CCCATGTCTC    |                       |

BseRI  
~~~~~

361	N L Y F Q S	
	AACCTGTACT TCCAATCCAT AAGCTAGCTT CTCCTCCTGA AAGATCCATA ACTTCGTATA	
	TTGGACATGA AGGTTAGGTA TTCGATCGAA GAGGAGGACT TTCTAGGTAT TGAAGCATAT	

- - - - - **SacB** - - - - - CGAGGAGTTT -2400

GCTCCTCAA

~~~~~

*BseRI*

#### Lic3

~~~~~

2401 ACTAGTAAGT AAAGGTGGAT ACGGATCCGA  
TGATCATTCA TTTCCACCTA TGCCTAGGCT

#### Primers for LIC cloning:

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

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

**pFB-6HZB sequence:**

atcatggagataattaaaatgataaccatctcgcaaaataataagtatttactgtttcgtaacagttt  
ttgtataaaaaaaacctataatattccggattattcataccgtcccaccatcggcgccgatctcggt  
ccgaaaaccatgggccaccatcatcatcattcttctgggtggataacaagttcaacaaggagcgt  
cgaagagctcgccgtgaaattcgccatctgccgaacctgaaccgcgaacacgcgtcgccattattcgc  
agcctgcgcgtatccgagccagagcgcgaacctgctggcggaagcgaagaagactgaacgatgcgcag  
ccgaagggtacagagaacctgtacttcaatccataagcttagttctccctgaaagatccataactt  
cgtatacgatacattatacgaagttatgcggccgcacgtccacatatacctgcgcgttactattatt  
agtgaardatgatattatgatatttctgaatttgatttttttttttttttttttttttttttttttttttttt  
aactataaaaaatacagagaatgaaaagaaacagatacgatgatatttttttttttttttttttttttttt  
aatccttttatgatattctatcaaacaaaagagggaaaatagaccagttcaatccaaacgagagtctaa  
tagaatgaggtcgaaaagtaatcgcgcgggttttttttttttttttttttttttttttttttttttttttt  
aaggggcaaaagtgtatactttggcgtcaccccttacatatttttttttttttttttttttttttttttt  
cttgcacatcttcaaacaggaggctggaagaagcagacccgctaaccacagatcataaaaaaggagacatg  
aacgatgaacatcaaaaagttgcacaaacaaagcaacagtttactaccgcactgtggcagg  
aggcgcaactcaagcgtttgcgaaagaaaacgaacccatataaggaaacatacggcatttttttttttt  
tattacacgcacatgatgtcacaatccctgaaacagaaaaatgaaaaatataaagttcctgagtt  
cgattcgtccacaatttttttttttttttttttttttttttttttttttttttttttttttttttttttttt  
aaacactgacggcactgtcgcacactatcacggctaccacatcgtcttcattagccggagatcctaa  
aatgcggatgacacatcgatttacatgttctatcaaaaagtcggcgaaacttcttattgacagctggaa  
aaacgctggccgcgtttaaagacagcgcacaaattcgatgcaatgattctatcctaaaagaccacaaac  
acaagaatggtcaggttcagccacatttacatctgcggaaaaatccgtttttttttttttttttttttt  
cggttaaacattacggcaaaacaaacactgacaaactgtcgcacactgttgcatttttttttttttttt  
gaacatcaacggtgttagaggattataatcaatcttgcgggtgacggaaaaacgtatcaaaatgttaca  
gcagttcatcgatgaaggcaactacagctcaggcgacaccatacgctgagagatcctactacgtaga  
agataaaaggccacaaatacttagtatttgcggactgttgcggactgttgcggactgttgcggactgtt  
atctttatccaacaaagcatactatggcaaaagcacaatcattttccgtcaagaaagtcaaaaacttct  
gcaaaagcgataaaaaacgcacggctgatgttgcggactgttgcggactgttgcggactgttgcggact  
ttacacactgaaaaaaagtgtatgaaaccgctgatgttgcggactgttgcggactgttgcggactgtt  
gaacgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgtt  
cgcatcgtctaaacgatatttacatgttgcggactgttgcggactgttgcggactgttgcggactgtt  
gctgaacaaaaactggcctgttttttttttttttttttttttttttttttttttttttttttttttttttt  
cttcgcgttacctaagcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
cgcagacacaaatacgcgttgcggactgttgcggactgttgcggactgttgcggactgttgcggact  
caaagacacatcccttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
tcctattggcatttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
cgaattcggactccgtcgacaaagctgtcgagaagactgttgcggactgttgcggactgttgcggact  
tagagtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt  
ttgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
caaataaaacgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
tctggatctgtatgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
ttgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
ctaaataatcccttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
gcattttcttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgtt  
gctacttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt  
tgaatatcaacgcgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
gcgggtgtgggttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
ttcttccttccttccttccttccttccttccttccttccttccttccttccttccttccttccttcct  
gggttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
ggccatcgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
ttgttccaaactggacaaacactcaaccatctcggttgcggactgttgcggactgttgcggactgttgcgg  
atttcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggact  
acgtttacaatttcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggact  
atacattcaaaatgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg  
aagagatgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggact  
tttgctcaccggactgttgcggactgttgcggactgttgcggactgttgcggactgttgcggactgtt  
atcgaactggatctcaacagcggactgttgcggactgttgcggactgttgcggactgttgcggactgtt  
agcactttaaagttctgtatgtggcggttgcggactgttgcggactgttgcggactgttgcggactgtt  
cgccgactattctcagaatgactgttgcggactgttgcggactgttgcggactgttgcggactgttgcgg

ggcatgacagtaagagaattatgcagtgcgcataaccatgagtgataacactgcggccaacttactt  
ctgacaacgatcggaggaccgaaggagctaaccgcgttttcacaacatggggatcatgtaactcgc  
cttgatcggtggaaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgta  
gcaatggcaacaacggtgcgcaaactattaactggcgaactactacttagcttccggcaacaatta  
atagactggatggaggcggataaagttgcaggaccacttctgcgctggcccttcggctggctgggtt  
attgctgataaacttggagccggtgagcgtgggtctcgccgtatcattgcagcactggggccagatgg  
aagccctcccgatcgttagttatctacacgacgggagtcaggcaactatggatgaaacgaaatagacag  
atcgctgagataggtgcctactgattaaagtttttttttttttttttttttttttttttttttttttttt  
tagattgattaaaacttcatttttttttttttttttttttttttttttttttttttttttttttttttttt  
accaaaatcccttaacgtgagtttcgttccactgagcgtcagaccccgtagaaaaagatcaaaggatct  
tctttagatccttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt  
gtttgttgcggatcaagagctaccaactttttccgaaggtaactggcttcagcagagcgcagata  
ccaaatactgtccttcttagtgttagccgttagtttaggccaccactcaagaactctgttagcaccgcctaca  
tacctcgctctgctaattcctgttaccagtggctgtgcgcgtggcgataagtctgttaccgggttgc  
gactcaagacgatagttacccggataaggcgcagcggcgtggctgaacgggggttcgtgcacacagccc  
agcttggagcgaacgacacctacaccgaactgagatacctacagcgtgagcattgagaaagcggcacgctt  
cccgaaaggagaaaggcggacaggatccggtaagcggcagggtcggaacaggagagcgcacgaggag  
cttccagggggaaacgcctggtatcttatagtctgtcggtttcgccacctctgactttagcgtcga  
ttttgtgatgctcgtcaggggggcgagcctatggaaaaacgcgcagcaacgcggccttttacggttc  
ctggcctttgtggcctttgtcacatgttcttcgttcatccctgatccctgattctgtggataaccgt  
attaccgccttgagttagtgcgtgataccgcctgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
gaggaagcggaaagagcgcctgtatggcgttgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
ccagccgcgtaacctggcaaaatcggttacggttagtaataatggatgcctgcgtaaagggtgt  
ggcggacaataaagtcttaactgaacaaaatagatctaaactatgacataaaagtcttaactagaca  
gaatagtgtaaactgaaatcgtccagttatgtgtgaaaaagcataactggactttttttatggctaa  
agcaaaactcttcatttctgaagtgcacattgcccgttatataagagggcggtggccaagggcatgg  
taaagactatattcggcgttgcacaattaccgaacaactccgcggccggaaagccgatctcggt  
tgaacgaatttttaggtggcggtacttgggtcgatataacttgcacacttcttccgtatgcccac  
tttgcgtatagagagccactgcgggatcgtcaccgtatctgtcgacgtagatcacataagcacaagg  
gcgttggcctatgcgttgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
actgcgagatcatagatagatctactacgcggctgtctcaaacctggcagaacgtaagccgcaga  
gcgcacaacaaccgccttgcgtcgaggcagcaagcgcgtatgttactacggagcaagttcccg  
aggtaatcggagtcggctgtatgttggagtaggtggctacgtctccgaactcacgaccgaaaagatca  
agagcagccgcatggatttgcgttgcggcgttgcggccgagcctacatgtgcgtatgcgcgc  
ccacctaacttttttagggcgactgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
gctccataacatcaaacatcgaccacggcgtaacgcgcgttgcgttgcgttgcgttgcgttgcgt  
tacaaaaaaacagtcatcataacaagccatgaaaaccgcactgcgcgttaccaccgcgttgcgt  
ggttctggaccagtgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
caactgggttcgtgccttcatccgtttccacggtgtgcgtcaccggcaaccttggcagcagcgaagt  
cgaggcatttctgtcctggctggcgaaacgagcgcgaagggttcgttgcgttgcgttgcgttgcgt  
ggccttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tcggccgtcgccggcgcttgcgggtgggtgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
aggcgagcatcggttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgt  
tattaatag

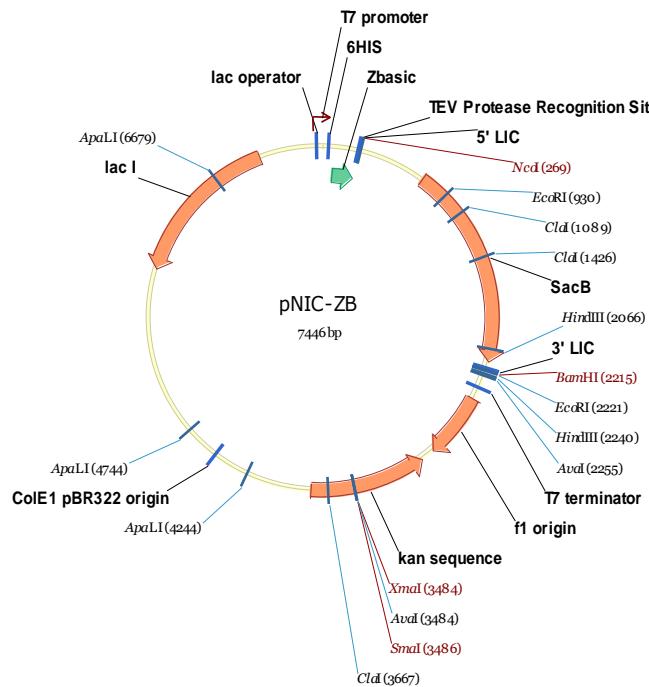
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pNIC-ZB</b>
Source	Pavel Savitsky
Sequence accession/link	Genbank GU452710

Description	pET expression vector with His <sub>6</sub> and Z-basic (ZB) tags followed by a TEV protease cleavage site. The Z-basic tag ( <i>J. Chromatog A</i> , 1161:22-28) is a 54-aa sequence derived from protein A and modified to have a high positive surface charge, allowing the fusion proteins to bind to S-sepharose at salt concentrations in which most cellular proteins do not bind. The vector includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIR SLRDDPSQSANLLAEAKKLNDDAQPKGTEONLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	8975 Da
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



### Polylinker region:

6HIS

~~~~~

NdeI

~~~~~

M H H H H H H H .

1 CTAGAAATAA TTTGTTAA CTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC  
GATCTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG  
6HIS  
Zbasic

~~ ~~~~~

· S S G V D N K F N K E R R R A R R E I R .

61 ATTCTCTGG TGTGGATAAC AAGTCAACA AGGAGCGTCG AAGAGCTCGC CGTGAAATTC  
TAAGAAGACC ACACCTATTG TTCAAAGTTGT TCCTCGCAGC TTCTCGAGCG GCACTTAACG  
Zbasic

~~~~~

· H L P N L N R E Q R R A F I R S L R D D .

121 GCCATCTGCC GAACCTGAAC CGCGAACAGC GTCGCGCATT TATTCCGAGC CTGCGCGATG  
CGGTAGACGG CTTGGACTTG GCGCTTGTG CAGCGCGTAA ATAAGCGTCG GACCGCGCTAC  
Zbasic

~~~~~

· P S Q S A N L L A E A K K L N D A Q P K .

181 ATCCGAGCCA GAGCGCGAAC CTGCTGGCGG AAGCGAAGAA GCTGAACGAT GCGCAGCCGA  
TAGGCTCGGT CTCGCGCTTG GACGACCGCC TTCGCTTCTT CGACTTGCTA CGCGTCGGCT  
5' LIC

~~~~~

Zbasic TEV Protease Recognition Site

~~ ~~~~~

NcoI

~~~~~

· G T E N L Y F Q S M

241 AGGGTACCGA GAACCTGTAC TTCCAATCCA TG  
TCCCAGGCT CTTGGACATG AAGGTTAGGT AC

### (SacB fragment)

EcoRI

BsaI Lower-LIC BamHI ~~~~~ SacI

~~~~~ ~~~~~ ~~~~~ ~~~~~

2010 GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTGAGCTC

CTATAGGATA ACCGTAAC TG CCAGAGGTCA TTTCCACCTA TG CCTAGGCT TAAGCTCGAG  
 Sali  
 HindIII  
 \*\*\*\*\*~~~~~~  
 2070 CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACCCAC CACCACTGAG ATCCGGCTGC  
 GCAGCTGTTG GAACGCCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCCGACG  
 ←-----  
 T7-reverse  
 2130 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCATA  
 ATTGTTCCGG GCTTCCCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT  
 ←-----  
 pLIC-rev

### Primers for LIC cloning:

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

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

pNIC-ZB sequence:

ctaaaaataattttgtttaacttaagaaggagatatacatatgcaccatcatcatcatcattttctg  
gtgtggataacaagttcaacaaggagcgtcgaaagagctgcgcgtgaaattgcgcattgcgcgaacctgaa  
accgcgaacacgcgtcgccatttattcgcagcgcgtatccgagccagcgcaacctgctgg  
cggaagcgaagaagctgaacgatgcgcagccgaagggtaccgagaacctgtacttcaatccatggaga  
ccgacgtccacatatacctggcgttactattatttagtgaaatgagatattatgatatttctgaatt  
gtgattaaaaggcaactttatgccatgcaacagaaaactataaaaatacagagaatgaaaagaaaca  
gatagatttttagttctttaggcccgtagtctgcaatcctttatgatttctatcaaacaagaaagag  
gaaaatagaccagtcaatccaaacgagagtctaatagaatgaggtcgaaaagttaatcgccgggtt  
tgttactgataaagcaggcaagacctaataatgtgtaaaggcaagtgtataacttgcgtcaccctt  
acatattttaggtcttttattgtcgtaactaacttgcatttcaaacaggagggtctgaaagaag  
cagaccgctaacacagtacataaaaaggagacatgaacgatgaacataaaaagttgcaaaacaagc  
aacagtattaaccttactaccgcactgctggcaggaggcgcaactcaagcgttgcgaaagaaacgaa  
ccaaaagccatataaggaaacatacggcatttccatattacacgcattgatagctgcaatccctga  
acagcaaaaaatgaaaatatacaagttcctgattcgattcgccacaattaaaatatcttctgc  
aaaaggcgtggacggtttggacagctggcattacaaaacgctgacggcactgtcgcaaaactatcacgg  
ctaccacatcgcttgcattagccggagatctaaaatcggtgacacatcgatttacatgttcta  
tcaaaaagtccggaaacttctattgacagctggaaaaacgctggccgcgtctttaagacagcgacaa  
attcgatgcaatgattctatctaaaagaccaacacaagaatggtcaggttcagccacattacatc  
tgacggaaaatcgttattctacactgattctccgtaaacattacggcaacaaacactgacaac  
tgcacaagttacgtatcagcatcagacagcttgcattacacggttagaggattataatcaat  
cttgcgggtacggaaaacgtatcaaaaatgtacagcgttcatcgatgaaaggcaactacagctcagg  
cgacaaccatacgtgagagatcctcactacgtagaagataaaggccacaaatacttagtatttgaagc  
aaacactggactgaagatggctaccaaggcgaagaatcttatttacaaagcatactatggcaaaag  
cacatcatttccgtcaagaaagtcaaaaacttgcacagcataaaaaacgcacggctgagttac  
aaacggcgctcggatgattgagctaaacgatgattacacactgaaaaagtgtatgaaaccgctgat  
tgcacatctaacacagtacacgatgaaattgacgcgcgacgtttaaatgaaacggcaatggtacct  
gttcactgactcccgcgatcaaaaatgacgattgcggcattacgtctaacgatattacatgcttgg  
ttatgttctaattttactggccatataaggcgtgaaacaaaactggctgtgttaaaaatgg  
tcttgatcctaacgatgtacacccattactcacacttcgtgtacctcaacgcgaaaggaaacaatgt  
cgtagattacaagctatgtacaaacagaggattctacgcagacaaacaatcaacggttgcgcgcaagctt  
cctgctgaaacatcaaaggcaagaaaacatctgttcaagacagcatccttgcacaaaggacaattaac  
agttacaaataaaaacgcacaaagaaaatgcccatacttgcgttgcacggctccagtaaggt  
gatacggatccgaattcgagctccgtcgacaagcttgcggccgcactcgagcaccaccaccacc  
tgagatccggctgtaacaaagccgaaaggaagctgagttggctgtgcaccgcgtgacataacta  
gcataacccttgggcctctaaacgggtctgaggggttttgcgtgaaaggaggactatatccgga  
ttggcgaatgggacgcgcctgtagcggcgttacgcgcgggtgtgggttacgcgcacgcgtga  
ccgctacacttgcagcgcctagcgcgcgtcattgcgttcttcccttctgcgcacgttgc  
ccggcttcccgtaagcttaatcgggggctccctttagggttgcatttagtgcatttacggcacc



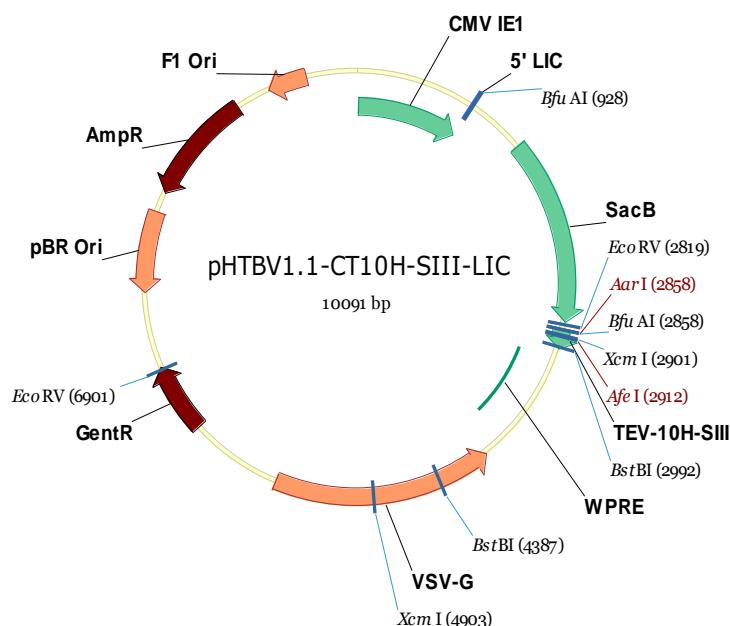
gactgttgccggccagtttgtgccacgcgggtggaatgtattcagctccgc  
acttttccccgcgtttcgagaaacgtggctggctggttcaccacgcggaa  
acaccggcatactctgcgacatcgatataacgttactggttcacatt  
tccggcgctatcatgccataccgcgaaagggttgcgcattcgatgg  
tcccttatgcgactcctgcatttaggaagcagccc  
aaggaatggtgcatgcaaggagatggccccaac  
gccgaaacaaggcgc  
gatgtcg  
gatata  
ggcgc  
cagcaaccgc  
cac  
ctgtggcc  
gggtgat  
gcgc  
ccac  
gtgc  
gtccgg  
cgat  
ggatcg  
gat  
atcc  
ctcgat  
ccc  
gcga  
atta  
at  
ac  
tgc  
act  
act  
at  
agg  
ggat  
ttgt  
gag  
cgata  
aca  
att  
ccc  
ct

Vector information sheet.

|                         |                                |
|-------------------------|--------------------------------|
| Vector Name             | <b>pHTBV1.1-CT10H-SIII-LIC</b> |
| Source                  | Claire Strain-Damerell         |
| Sequence accession/link | (SGC)                          |

|             |   |
|-------------|---|
| Description | Baculovirus transfer vector for expression of proteins in mammalian cells, with C-terminal His <sub>10</sub> tag and SIII tag, preceded by a TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose. The vector also has full length VSVG for pseudotyping of the baculovirus. |
|-------------|---|

|                          |  |
|--------------------------|--|
| Antibiotic resistance    | Ampicillin, 100 µg/ml  |
| Promoter                 | CMV with synthetic intron  |
| Cloning                  | LIC (vector treated with BfuAI, then with T4 DNA polymerase in presence of dCTP)   |
| Initiation codon         | Supplied in PCR primer   |
| C-terminal fusion – seq. | AENLYFQ*SHHHHHHHHHGSAWSHPQFEKGGGSGGSG (* - TEV cleavage site)  |
| C-terminal fusion – MW   | 5426.68 Da   |
| Termination codon        | Downstream of SIII tag   |
| Protease cleavage        | TEV (removes 4560.74 Da)   |
| Additional features      | Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).   |
| Preferred host           | Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA. Bacmid DNA can be transfected to insect cells to generate recombinant baculovirus. Baculovirus can be used to produce recombinant protein in multiple mammalian cell lines. |
| 5' sequencing primer     | pFBM-fwd caaaaatgtcgtaacaactccgc   |
|                          | pFBM-rev tagttagaataaccagtcaatcttcac   |



Cloning region in the vector:

5' end:

5' LIC  
~~~~~  
BfuAI  
~~~~~

901 TCGAGCTCAA GCTTCTTAAG AAGGAGATAT ACTATGCAGG TCGTTCACTA TTATTTAGTG  
AGCTCGAGTT CGAAGAACCC TTCCTCTATA TGATACGTCC AGCAAGTGAT AATAAAATCAC

----- SacB fragment -----

TEV  
~~~~~  
3' LIC  
~~~~~  
BfuAI  
~~~~~

2821 CCTATTGGCA TTGACGTCAG GTGGCACACC TGCAGAGAAC CTCTACTTCC AATCGCACCA  
GGATAACCGT AACTGCAGTC CACCGTGTGG ACGTCTCTTG GAGATGAAGG TTAGCGTGGT

10 His  
~~~~~

2881 · H H H H H H H G S A W S H P Q F E K G ·  
TCATCACCAC CACCATCACC ACCATGGCAG CGCTTGGAGC CACCCGCAGT TCGAGAAAGG  
AGTAGTGGTA GTGGTAGTGG TGGTACCGTC GCGAACCTCG GTGGCGTCA AGCTCTTCC

SIII  
~~~~~

2941 · G G S G G G S G G S A W S H P Q F E K \* ·  
TGGAGGTTC GGAGGTGGAT CGGGAGGTTC GGCCTGGAGC CACCCGCAGT TCGAAAAATG  
ACCTCCAAGG CCTCCACCTA GCCCTCCAAG CCGCACCTCG GTGGCGTCA AGCTTTTAC

· \*

3001 ATCTAGATCA  
TAGATCTAGT

Primers for LIC cloning:

Add the following 5' extensions to the PCR primers:

Upstream: TTAAGAAGGAGATATACTATG (ATG-initiation codon)

Downstream: GATTGGAAGTAGAGGTTCTCTGC

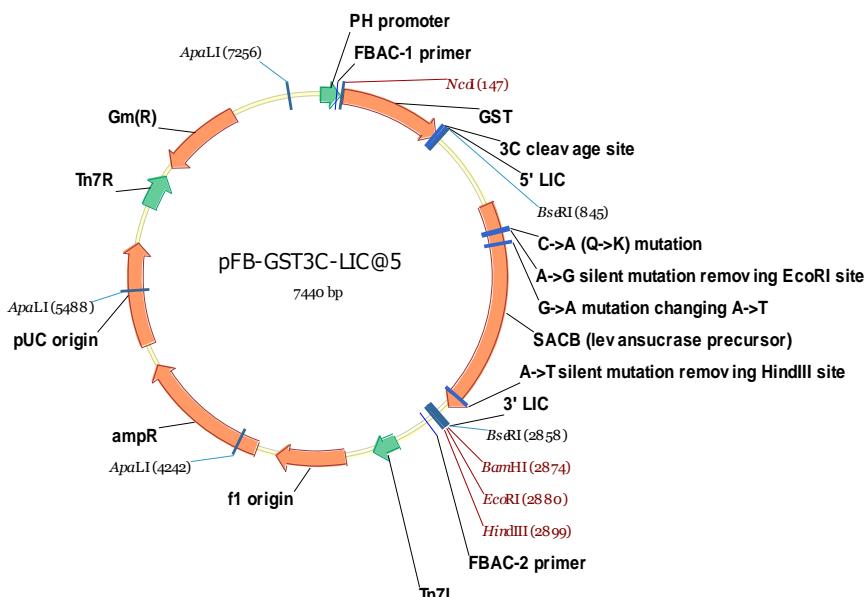
The purified PCR fragments are treated with T4 DNA polymerase and dGTP, then annealed to the treated vector.

# Vector information sheet

Dated: 27<sup>th</sup> January 2015

Vector Name	<b>pFB-GST3C-LIC</b>
Source	Claire Strain-Damerelli
Sequence accession/link	(SGC)

Description	Baculovirus transfer vector with GST tag in 232-aa N-terminal fusion peptide, with 3C protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection of transformed bacteria on 5% sucrose
Antibiotic resistance	Ampicillin, 100 µg/ml
Promoter	Polyhedrin
Cloning	LIC (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGSPILGYWKIKGLVQPTRLLEEKYEEHLYERDEGDKWWRNKKFELGLEF PNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGAVIDIRYG VSRIAYSKDFETLKVDLFLSKPEMLKMFDRLCHKTLYNGDHVTHPDFMLYD ALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLEVLFQ*GPLSM (* - 3C cleavage site)
N-terminal fusion – MW	26973.5 Da including Met
Termination codons	supplied in PCR primer
Protease cleavage	3C
Additional features	Tn7 sequences for in vivo recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA
5' sequencing primer	FBAC1: TATTCACTACCGTCCCACCA
3' sequencing primer	FBAC2: GGGAGGTTTTAAAGCAAGTAAA



Cloning region in the vector:

	NcoI
	~~~~~
	M G S P I L G Y W K I
121	TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCTCCCCTA TACTAGGTTA TTGGAAAATT AGCCCGCGCC TAGAGCCAGG CTTTGGTAC CCGAGGGAT ATGATCCAAT AACCTTTAA K G L V Q P T R L L L E Y L E E K Y E E
181	AAGGCCCTG TGCAACCCAC TCGACTTCTT TTGGAATATC TTGAAGAAAA ATATGAAGAG TTCCCGAAC ACGTGTTGGTG AGCTGAAGAA AACCTTATAG AACTCTTT TATACTCTC H L Y E R D E G D K W R N K K F E L G L
241	CATTTGTATG AGCGCGATGA AGGTGATAAA TGGCAGAACAA AAAAGTTGA ATTGGGTTTG GTAAACATAC TCGCGCTACT TCCACTATTT ACCGCTTTGT TTTTCAAAC TAAACCCAAAC E F P N L P Y Y I D G D V K L T Q S M A
301	GAGTTTCCA ATCTCCTTA TTATATTGAT GGTGATGTTA AATTAACACA GTCTATGGCC CTCAAAGGGT TAGAAGGAAT AATATAACTA CCACTACAAT TTAATTGTT CAGATACCGG I I R Y I A D K H N M L G G C P K E R A
361	ATCATACGTT ATATAGCTGA CAAGCACAAAC ATGTTGGGTG GTTGTCCAAA AGAGCGTGCA TAGTATGCAA TATATCGACT GTTCGTGTTG TACAACCCAC AACAGGTTT TCTCGCACGT E I S M L E G A V L D I R Y G V S R I A
421	GAGATTCAA TGCTGAAGG AGCGGTTTG GATATTAGAT ACGGTGTTC GAGAATTGCA CTCTAAAGTT ACGAACTTCC TCGCCAAAAC CTATAATCTA TGCCACAAAG CTCTAACGT Y S K D F E T L K V D F L S K L P E M L
481	TATAGTAAAG ACTTGAAAC TCTCAAAGTT GATTTCTTA GCAAGCTACC TGAAATGCTG ATATCATTTC TGAAACTTTG AGAGTTCAA CTAAAAGAAT CGTTCGATGG ACTTTACGAC K M F E D R L C H K T Y L N G D H V T H
541	AAAATGTTCG AAGATCGTTT ATGTCATAAA ACATATTAA ATGGTGTCA TGTAACCCAT TTTTACAAGC TTCTAGCAAA TACAGTATTT TGTATAAATT TACCACTAGT ACATTGGTA P D F M L Y D A L D V V L Y M D P M C L
601	CCTGACTTCA TGTTGTATGA CGCTCTTGAT GTTGTGTTTACATGGACCC AATGTGCCTG GGACTGAAGT ACAACATACT GCGAGAACTA CAACAAAATA TGTACCTGGG TTACACGGAC D A F P K L V C F K K R I E A I P Q I D
661	GATGCCTTCC CAAAATTAGT TTGTTTTAAA AAACGTATG AAGCTATCCC ACAAATTGAT CTACGCAAGG GTTTAATCA ACAAAATT TTTGCATAAC TTCGATAGGG TGTAACTA K Y L K S S K Y I A W P L Q G W Q A T F
721	AAGTACTTGA AATCCAGCAA GTATATAGCA TGGCCTTGC AGGGCTGGCA AGCCACGTT TTCATGAACT TTAGTCGTT CATATATCGT ACCGAAACG TCCCGACCGT TCGGTGCAAA G G G D H P P K S D L E V L F Q G P L S
781	GGTGGTGGCG ACCATCCTCC AAAATCGGAT CTGGAAGTC TGTTCCAGGG CCCACTCTCT CCACCACCGC TGGTAGGAGG TTTAGCCTA GACCTTCAAG ACAAGGTCCC GGGTGAGAGA BseRI ~~~~~
841	ATCCGCTAGC TTCTCCTCCT GAAAGATCCA TAACTCGTA TAGCATACAT TATACTGAAGT TAGGCATCG AAGAGGAGGA CTTCTAGGT ATTGAAGCAT ATCGTATGTA ATATGCTTC

----- SacB -----

BseRI	BamHI
~~~~~	~~~~~
2821	TGACGTCAGG TGGCACTTT CGAGGAGTTT ACTAGTAAGT AAAGGTGGAT ACGGATCCGA ACTGCAGTCC ACCGTGAAAA GCTCCTCAA TGATCATTCA TTTCCACCTA TGCCTAGGCT

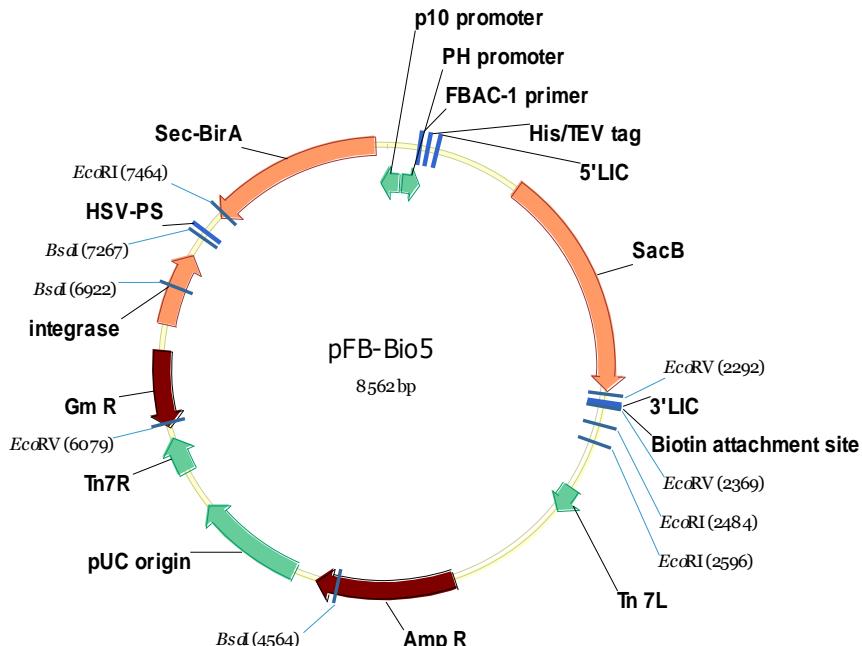
Primers for LIC cloning:

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

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

Vector information sheet.

Vector Name	<b>pFB-Bio5</b>
Source	Opher Gileadi and Claire Strain-Damerell
Sequence accession/link	
Description	Baculovirus transfer vector with His <sub>6</sub> tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site and C-terminal biotin attachment site. The vector also includes a secreted version of <i>birA</i> under the control of the p10 promoter for <i>in vivo</i> biotinylation. Includes sites for LIC cloning, and a "stuffer" fragment that includes the <i>SacB</i> gene, allowing negative selection of transformed bacteria on 5% sucrose.
Antibiotic resistance	Ampicillin, 100 µg/ml
Promoter	Polyhedrin
Cloning	LIC. (vector treated with BseRI, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MGHHHHHHHSSGVDLGTENLYFQ*SM (* - TEV cleavage site)
N-terminal fusion – MW	2630 Da including Met (2411.8 Da removed by TEV cleavage)
C-terminal fusion – seq.	SSKGYYGLNDIFEAQKIEWHE
C-terminal fusion – MW	2408.6 Da
Termination codons	supplied in PCR primer
Protease cleavage	TEV (cleaves His-tag only)
Additional features	Tn7 sequences for <i>in vivo</i> recombination into bacmid DNA in DH10Bac (using InVitrogen's Bac-to-bac system).
Preferred host	Initial transformation into any cloning strain, then transform purified plasmid into DH10Bac to generate recombinant bacmid DNA
5' sequencing primer	FBAC1: TATTCACTACCGTCCCACCA
3' sequencing primer	FBAC2: GGGAGGTTTTAAAGCAAGTAAA



Opher Gileadi and Claire Strain-Damerell, Structural Genomics Consortium (SGC), Oxford. Email: opher.gileadi@sgc.ox.ac.uk / claire.strain-damerell@sgc.ox.ac.uk

Cloning region in the vector:

```

Fbac1
----->
181 AATAAAAAAA CCTATAAATA TTCCGGATTA TTCATACCGT CCCACCATCG GGCGCGCTTA
TTATTTTTT GGATATTAT AAGGCCTAAT AAGTATGGCA GGGTGGTAGC CCGCGCGAAT

M G H H H H H S S G V D L G
241 AGAAGGAGAT ATACTATGGG CCACCATCAT CATCATCATT CTTCTGGTGT AGATCTGGGT
TCTTCCTCTA TATGATAACCC GGTGGTAGTA GTAGTAGTAA GAAGACCACA TCTAGACCCA
BseRI
~~~~~
T E N L Y F Q
301 ACCGAGAACCC TGTACTTCCA ATCCATAAGC TAGCTTCTCC TCCTGAAAGA TCCATAACTT
TGGCTCTTGG ACATGAAGGT TAGGTATTCG ATCGAAGAGG AGGACTTCT AGGTATTGAA
LIC5'

```

--SacB linker--

```

BseRI
~~~~~
2281 AAAATGCCGA TATCCTATTG GCATTGACGT CAGGTGGCAC TTTTCGAGGA GTTTACTAGT
TTTACGGCT ATAGGATAAC CGTAACTGCA GTCCACCGTG AAAAGCTCCT CAAATGATCA

S K G G Y G L N D I F E A Q K I E W H E .
2341 AAGTAAAGGT GGATACGGCC TGAATGATAT CTTTGAAGCG CAGAAGATTG AATGGCATGA
TTCATTTCCA CCTATGCCGG ACTTACTATA GAAACTTCGC GTCTTCTAAC TTACCGTACT
LIC3'

. * *
2401 ATGATAAGCA GAGAACCTCT ACTTCCAATC GGGTCTGAAC GACATCTCG AAGCTCAGAA
TACTATCGT CTCTGGAGA TGAAGGTTAG CCCAGACTG CTGTAGAACG TTGGAGTCTT

2461 AATTGAATGG CACTGAGGAT CCGAATTGCA GCTCCGTCGA CAATTGGCA GAGAACCTCT
TTAACTTACC GTGACTCCTA GGCTTAAGCT CGAGGCAGCT GTTAACCCGT CTCTGGAGA

2521 ACTTCCAATC GCACCATCAT CACCATCACC ATCACCAAG TGATTACAAG GATGACGACG
TGAAGGTTAG CGTGGTAGTA GTGGTAGTGG TAGTGGTGGT ACTAATGTT CTACTGCTGC
FBac2
-----<-----
2641 ATAATCAGCC ATACCACATT TGTAGAGGTT TTACTTGCTT TAAAAAACCT CCCACACCTC
TATTAGTCGG TATGGTGTAA ACATCTCAA AATGAACGAA ATTTTTGGA GGGTGTGGAG

```

Primers for LIC cloning:

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

Downstream: add TATCCACCTTACTGCT to 5' end of downstream primer. Do not add termination codon as this will remove the biotin attachment site.

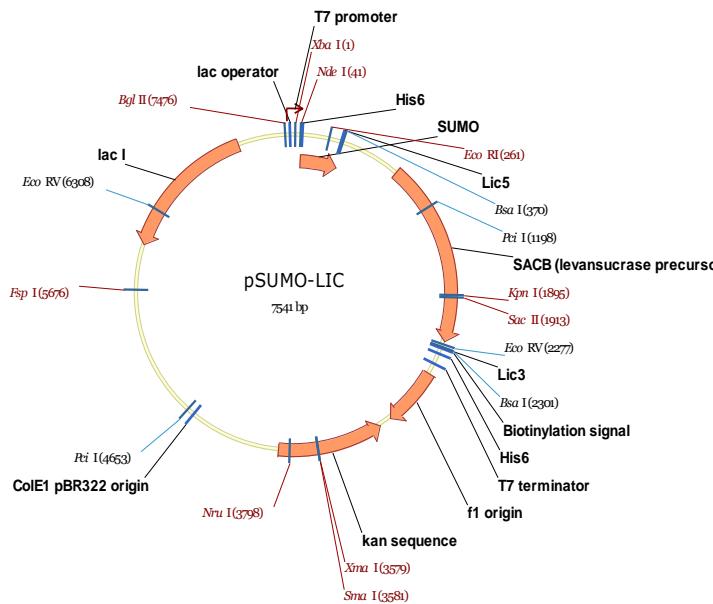
# Vector information sheet

Dated: 16<sup>th</sup> September 2013

Vector Name	<b>pSUMO-LIC</b>
Source	Pavel Savitsky
Sequence accession/link	Genbank XXXXXXXX

Description	pET expression vector with His <sub>6</sub> and SUMO (96-aa sequence derived from SUMO1 protein) tags followed by a SUMO protease cleavage site. SUMO tag has been shown to improve solubility of the fusion partners. The vector includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose. If no stop codon is included in reverse primer, protein of interest will be fused to biotinylation tag followed by additional His <sub>6</sub> tag.
-------------	------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dTTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MCSSHHHHHHGSGSGSDQEAKPSTEDLGDKKEGEYIKLKVG QDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRI ADNHTPKELGMEEEDVIEVYQEQTGG* (* - SUMO cleavage site)
N-terminal fusion – MW	12577.9 Da
Termination codons	supplied in PCR primer
Protease cleavage	SUMO
Additional features	Optional non-cleavable biotinylation and His <sub>6</sub> tags if reverse primer does not encode stop codon.
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



Cloning region in the vector:

	XbaI	NdeI
1	CTAGAAATAA TTTTGTAA CTTTAAGAAG GAGATATACA TATGTGCAGC AGCCATCATC GATCTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACACGTG TCGGTAGTAG SUMO	M C S S H H H H
61	H H H G S G S G S D Q E A K P S T E D L ATCATCATCA CGGCAGCGGC AGCGGCTCTG ACCAGGAGGC AAAACCTTCA ACTGAGGACT TAGTAGTAGT GCCGTCGCCG TCGCCGAGAC TGGTCTCCG TTTTGAAGT TGACTCCGTA SUMO	~~~~~
121	G D K K E G E Y I K L K V I G Q D S S E TGGGGGATAA GAAGGAAGGT GAATATATTA AACTCAAAGT CATTGGACAG GATAGCAGTG ACCCCCTATT CTTCCTTCCA CTTATATAAT TTGAGTTCA GTAACCTGTC CTATCGTCAC SUMO	~~~~~
181	. I H F K V K M T T H L K K L K E S Y C Q AGATTCACTT CAAAGTGAAA ATGACAACAC ATCTCAAGAA ACTCAAAGAA TCATACTGTC TCTAAGTGAA GTTCACTTT TACTGTTGTG TAGAGTTCTT TGAGTTCTT AGTATGACAG SUMO	~~~~~
241	. R Q G V P M N S L R F L F E G Q R I A D AAAGACAGGG TGTCCAATG AATTCACTCA GGTTCTCTT TGAGGGTCAG AGAATTGCTG TTTCTGTCCC ACAAGGTTAC TTAAGTGAGT CCAAAGAGAA ACTCCCAGTC TCTTAACGAC SUMO	~~~~~
301	. N H T P K E L G M E E E D V I E V Y Q E ATAATCATAC TCCAAAAGAA CTGGGAATGG AGGAAGAAGA TGTGATTGAA GTTACCAAGG TATTAGTATG AGGTTTCTT GACCCTTACC TCCTTCTTCT ACACTAACTT CAAATGGTCC SUMO	~~~~~
361	. Q T G G G AGAACACGGG AGGT TGGTTTGCCTC TCCA	~~~~~

2401 AGGAAGCTGA GTTGGCTGCT GCCACCGCTG AGCAATAACT AGCATAACCC CTTGGGGCCT  
TCCTTCGACT CAACCGACGA CGGTGGCGAC TCGTTATTGA TCGTATTGGG GAACCCCCGGA

## Lic cloning scheme:

Treat vector with dTTP:

V Y Q E Q T G G BsaI  
 GTT ACCAGGAGCAAACGG GAGGT GAGACCC GACGTCCACATatacctggcggtactattttt  
 CAAATGGTCTCGTTGCCCTC ACTCTGGCTGCAGGTGTatatggacggcaagtqataataaa

(SacB fragment)

BsaI	S G G G L N D I F E A Q	
GATATCCTAT TGGCATTGAC	<b>GGTCTCC</b>	AGCGGTGGGGTCTGAACGACATCTTCGAGGCTCAG
CTATAGGATA ACCGTAACTG	<b>CCAGAGGTCGC</b>	CACCGCCAGACT <b>T</b> GTGTAGAAGCTCCGAGTC

K I E W H E H H H H H H  
AAAATCGAATGGCACGAACA CCACCAACCAC CACCACTTGAGATCCGGCTGC  
TTTTAGCTTACCGTGTGTTGT GGTGGTGGTG GTGGTGACTCTAGGCCGACG

Treat PCR product with dATP:

ACCAGGAGCAACGGGAGGT TGGTCCTCGTTGCCCTCC	PCR product	TGAAAGCGGTGGCGGTCTG AGTTCCGCCACCGCCAGAC
-------------------------------------------	-------------	--------------------------------------------

#### Primers LIC extensions:

Forward	ACCAGGAGCAAACGGGAGGT
Reverse1	CAGACCGCCACCGCT
Reverse2	CAGACCGCCACCGTTGA

Primers for LIC cloning (treat PCR fragment with T4 DNA polymerase in presence of dATP)

Upstream: add ACCAGGAGCAAACGGGAGGT to the 5' end of upstream primer

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

pSUMO-LIC sequence:

ctagaaataatttgtttaacttaagaaggagatatacatatgtcagcagccatcatcatcatcatcat  
acggcagcggcagcgctctgaccaggaggcaaacctcaactgaggacttgaaaaataagaaggaag  
gtgaatatattaaactcaaagtcatggacaggatagcagttagattcaacttcaaagtgaaaatgaca  
cacatctcaagaaactcaaagaatcatactgtcaaagacaggggtgttccaatgaattcactcaggttc  
tcttgagggtcagagaattgctgataatcatactccaaaagaactggaaatggaggaagaagatgtga  
ttgaagtttaccaggagcaaacgggaggtgagaccgcgtccacatatacctgccgttcaacttattt  
agtgaaaatgagatattatgatatttctgaattgtgattaaaaaggcaactttatgcccattgcaacaga  
aactataaaaaatacagagaatgaaaagaaacagatagatttttagttctttaggcccgtagtctgca  
aatcctttatgatttctatcaaacaaaagagggaaatagaccagtgtcaatccaaacgagagtctaa  
tagaatgaggtcggaaaagtaatcgcgcgggtttagttactgataaaggcaggcaagacctaaaatgtga  
aaggcggaaaatgtatactttggcgtcccccttacatatttttaggtcttttattgtcgtactaa  
cttgcctatcttcaaacaggaggcgttggaaaagcagaccgctaacacagtacataaaaaaggagacatg  
aacatgtgaaatcaaaaagttgcaaaacaagcaacagtattacccctactaccgcactgtggcagg  
aggcgcaactcaagcgttgcggaaaacgacccatataaggaaacatcggcatttcca  
tattacacgcattatgtgtcaatccctgaacacgcaaaaaatgaaaaatataaaagtctgtgatt  
cgattcgtccacaattaaaaatcttctgcaaaaggcctggacgttggacagctggcattaca  
aaacactgacggactgtcggaaaactatcaggctaccacatgtcttgcattagccggagatctaa  
aatgcccgtgacacatcgatttacatgttctatcaaaaagtcggcggaaaacttcttattgacagctggaa  
aaacgctggccgcgtttaagacagcgacaaattcgatgcaatgattctatctaaaagacacaaac  
acaagaatggtcaggttcagccacatttacatctgacggaaaatccgttattctacactgttctc  
cggttaaacattacggcaacaaaacactgacaactgcacaagttacgtatcagcatcagacagctt  
gaacatcaacgggttagaggattataatcaatcttgcacgggtacggaaaacgtatcaaaaatgtaca  
gcagtcatcgatgaaggcaactacagctcaggcgacaaccatacgtgagagatcctcactacgtaga

agataaaaggccacaaatacttagtatttgaagcaaacactggaactgaagatggctaccaaggcagaaga  
atctttatTTaacaAGcatactatggcaAAAGcacatcattttcgctcaAGAAAGTCAAATCT  
gcaaAGcgataAAAACGcAcGGCTGAGTTGAGCAACGGCCTCTCGGTATGATTGAGCTAAACGATGA  
ttacacactgaaaaAGTGTGAAACCGCTGATTGACTCTAACACAGTAACAGATGAAATTGACGCGC  
gaACGTCTTAAATGAACGGCAAATGGTACCTGTTACTGACTCCCGGGATCAAAATGACGATTGA  
CGGCATTACGTCTAACGATAATTACATGCTTGGTTATGTTCTAATTCTTAACGGCCATACAAGCC  
GCTGAACAAAACGGCCTGTTAAAATGGATCTTGACTCTAACGATGTAACCTTACTTCACA  
CTTCGCTGTACCTCAAGCGAAAGGAAACAAATGTCGTGATTACAAGCTATATGACAAACAGAGGATTCTA  
CGCAGACAAACAATCAACGTTGCGCTAGCTCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGT  
CAAAGACAGCATCCTGAACAAGGACAATTAAACAGTTAACAAATAAAACGCAAAGAAAATGCCGATA  
TCCTATTGGCATTGACGGTOTCCAGCGGTGGCGGTCTGAACGACATCTCGAGGCTCAGAAAATCGAAT  
GGCACGAAACACCACCAACCACTGAGATCCGGCTGCTAACAAAGGCCGAAAGGAAGCTGAGTTGG  
CTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTGGGGCCTCTAAACGGGTCTTGAGGGGTTTT  
TGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCGGCCGCTTAAGCGCGC  
GGGTGTGGTGGTTACGCGCAGCGTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTCGCTTT  
CTTCCTTCCTTCTGCCACCGTTGCGGGCTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTCTAGG  
GTTCCGATTTAGTGCTTAACGGCACCTGACCCAAAAACTTGATTAGGGTGTGGTTCACGTAGTGG  
GCCATCGCCCTGATAGACGGTTTCGCCCTTGACGTTGGAGTCCACGTTCTTAATAGTGGACTCTT  
GTTCCAACACTGGAACAACACTCAACCTATCTCGTCTATTCTTTGATTATAAGGGATTTGCCGAT  
TTCCGCTATTGGTTAAAATGAGCTGATTAAACAAATAACGCAATTAAACAAATATTAAAC  
GTTACAATTTCAGGTGGCACTTTGGGAAATGTGCGCGAACCCCTATTGTTATTCTAAAT  
ACATTCAAATATGTTACCGCTCATGAATTAAATTCTTAGAAAACATCGAGCATCAAATGAAACTGCA  
ATTATTCAATACAGGATTATCAATAACCATATTGAAAAGCCGTTCTGTAATGAAGGAGAAAAC  
CACCGAGGCGAGTCCATAGGATGGCAAGATCCTGTTCTGCGATTCCGACTCGTCCAACATCAA  
TACAACCTATTAAATTCCCTCGTCAAAAATAAGTTATCAAGTGAGAAAATCACCAGTGTGACGACTG  
AAATCCGTTGAGAATGGCAAAGTTATGCTTCCAGACTTGTTCAACAGGCCAGCATTACGCT  
CGTCACTAAAATCACTCGCATCAACAAACCGTTATTCTGTTGAGCGAGACAGAAATA  
CGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCCAGGAACACTGCCAGCG  
CATCAACAAATTTCCCTCGTCAAAATAAGTTATCGATCCATGTTGAGTCCACGTTCTGCGGAGATCG  
CAGTGGTGTGAGTAACCATGTCATCGAGGATAAAATGCTGTTGAGTGGCGAGAGGATAAATT  
CCGTAGGCCAGTTAGTCTGACCATCTCATCTGTAACATCATGGCAACGCTACCTTGCCATGTTCA  
GAAACAACCTGGCGCATGGGCTTCCACATCGATAGATTGTCGACCTGATTGCCGACATTAT  
CGCGAGGCCATTATAACCCATATAAAATCAGCATCCATGTTGAGATTAAATCGCGGCCAGAGCAAGACG  
TTTCCCCTGTTGAGATATGGCTCATACACCCCTGTATTACTGTTATGTAAGCAGACAGTTTATTGTC  
ATGACCAAAATCCCTAACGTTGAGTTCTGCTGAGCTGAGCCAGGTTGAGGCTACCGCTACCGAG  
TCTTCTGAGATCCTTTCTGCGGTAATCTGCTGTTGCAAACAAAAACCCCGCTACCGAG  
GTGGTTGTTGCCGGATCAAGAGCTACCAACTCTTTCCAGGTTAAGCTGGCTCAGCAGAGCGCAG  
ATACCAAATACTGTCCTCTAGTGTAGCGTAGTTAGGCCACCACTCAAGAACACTCTGTAAGCACC  
ACACATCTCGCTGCTAACCTGTTACCGACTGCTGCTGCGATAAGTCGTGTTACCGGG  
TTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGCTGAACGGGGGTTCTGTCACACAG  
CCAGCTTGGAGCGAACGACCTACACCGAACTGAGAGACCTACAGCGTGTAGCTATGAGAAAGCGCCACG  
CTTCCCAGGGAGAAAGGGGGACAGGTATCCGGTAAGCGGCAGGGTCCGAACAGGAGAGCGCAGG  
GAGCTTCCAGGGGGAAACGCGCTGGTACCTTATAGTCCTGCGGGTTCTGCCACCTCTGACTGAGCGT  
CGATTTGTGATGCTGTCAGGGGGCGGAGCCTATGAAAACGCCAGCAACCGCCCTTACGG  
TTCTGCGCTTTGCTGGCCTTGTCTCACATGTTCTGCGTTATCCCTGATTCTGTTGATAAC  
CGTATTACCGCCTTGAGTGTAGCTGATACCGCTCGCCAGCGAACAGCAGCGCAGCGAGTCAGTG  
AGCGAGGAAGCGGAAGAGCGCTGATGCGGTATTCTCCTACGCTACGCGTGTAGCTGAGGTTAC  
ATATATGGTGCACCTCTCGTACATCTGCTGATGCCGCTAGTTAAGCCAGTATAACACTCCGCTATC  
GCTACGTTGACTGGGTATGGCTGCCCGACACCCGCAACACCCGCTGACGCCCTGACGGGCTTG  
TCTGCTCCCGGACATCCGCTTACAGACAAGCTGTGACCGTCTCCGGAGCTGATGTCAGAGGTTTC  
ACCGTACATCACCAGAAACCGCGCAGGGCAGCTGCGGTAAGCTCATCGCTGGCTGTAAGCGATTACA  
GATGTCGCGCTGTACATCCGCGTCCAGCTGTTGAGTTCTCCAGAAGCGTTAATGTCGGCTTCTGAT  
AAAGCGGGCCATGTTAAGGGCGTTTCTGTTGAGTGTGACGTTGCTCCGCTGTAAGGGGATTCT  
GTTCATGGGGTAATGATAACGATGAAACGAGAGAGGATGCTCACGATAACGGGTTACTGATGATGAACA  
TGGCCGGTTACTGGAACGTTGAGGGTAACAAACTGGCGGTATGGATGCGGCCGGACAGAGAAAAT  
CACTCAGGGTCAATGCCAGCGCTCGTTAACACAGATGTTGAGGTTCCACAGGGTAGGCCAGCGAC  
TGCAGATGCGAGATCCGGAACATAATGGTCAGGGCGCTGACTTCGCGTTCCAGACTTACGAAACACG  
GAAACCGAAGACCAATTGTTGCTCAGGTCGAGACGTTGCGAGCGAGTCGCTCACGTTG  
CTCGCGTATCGGTGATTCTGCTAACCGAGTAAGGCAACCCGCCAGCCTAGCCGGTCTCAACGA  
CAGGAGCACGATCATGCGCACCCGTTGGGCCAGTGCAGGCGATAATGGCTGCTTCTGCGCAG  
TTTGGTGGCGGGACCGAGTGAACGAGGTTGAGCGAGGGCGTGAAGATTCCGAATACCGCAAGCGACAG

gccgatcatcgctcgccagcgaaagcggtcctcgccaaaaatgacccagagcgctgccggcacctg  
tcctacagttgcataaaaagaagacagtataagtgcggcgcacgatagtcatgccccgcgcccaccg  
gaaggagactgactgggttgaaggctctcaagggcatcggtcgagatccgggtgcctaattgagttagtgc  
acttacattaattgcgttgcgtactgcggcgttccagtcggaaacctgtcgccagctgcatta  
atgaatcgccaaacgcgcggggagaggcggttgcgtattggcgccagggtggtttttttccacca  
gtgagacggcaacagctgatgcctcaccgcctggccctgagagatgtcgagcaagcggtccacgc  
tggtttgcggcaggcgaaaatcctgtttgatgggttaacggcgggatataacatgagctgtctt  
cggtatcgctgtatcccactaccgagatatccgcaccaacgcgcagccggactcgtaatggcgca  
ttgcgcggcagcgccatctgatcggttgcacccagcategcagttggaaacgatgcctcattcagcattt  
gcatggtttgtgaaaaccggacatggcactccagtcgcctccgtatcgctgaatttgat  
tgcgagtgagatattatgcacccagcagacgcgcggagacagaacttaatggcccgcta  
acagcgcgatttgcgtggtacccaatgcgaccagatgctccacgcccagtcgcgtaccgtcttcatgg  
agaaaaataactgttgcgttgcgttgcagagacatcaagaaaataacgcggaaacatttagtgcagg  
cagctccacagcaatggcatcctggcatccagcgatagttaatgatcagcccactgacgcgttgc  
cgagaagattgtgcaccgcgcgttacaggcttcgcgcgttgcgttaccatcgacaccaccacgc  
tggcaccaggatgtgcgcgcgagatttaatgcgcgcgacaatttgcgcgcgtgcaggccagac  
tggagggtggcaacgc当地点的坐标是(100, 100)到(900, 900)。在这些坐标内，文本将被随机地分布在不同的位置，使得文本块看起来像是漂浮在空间中。

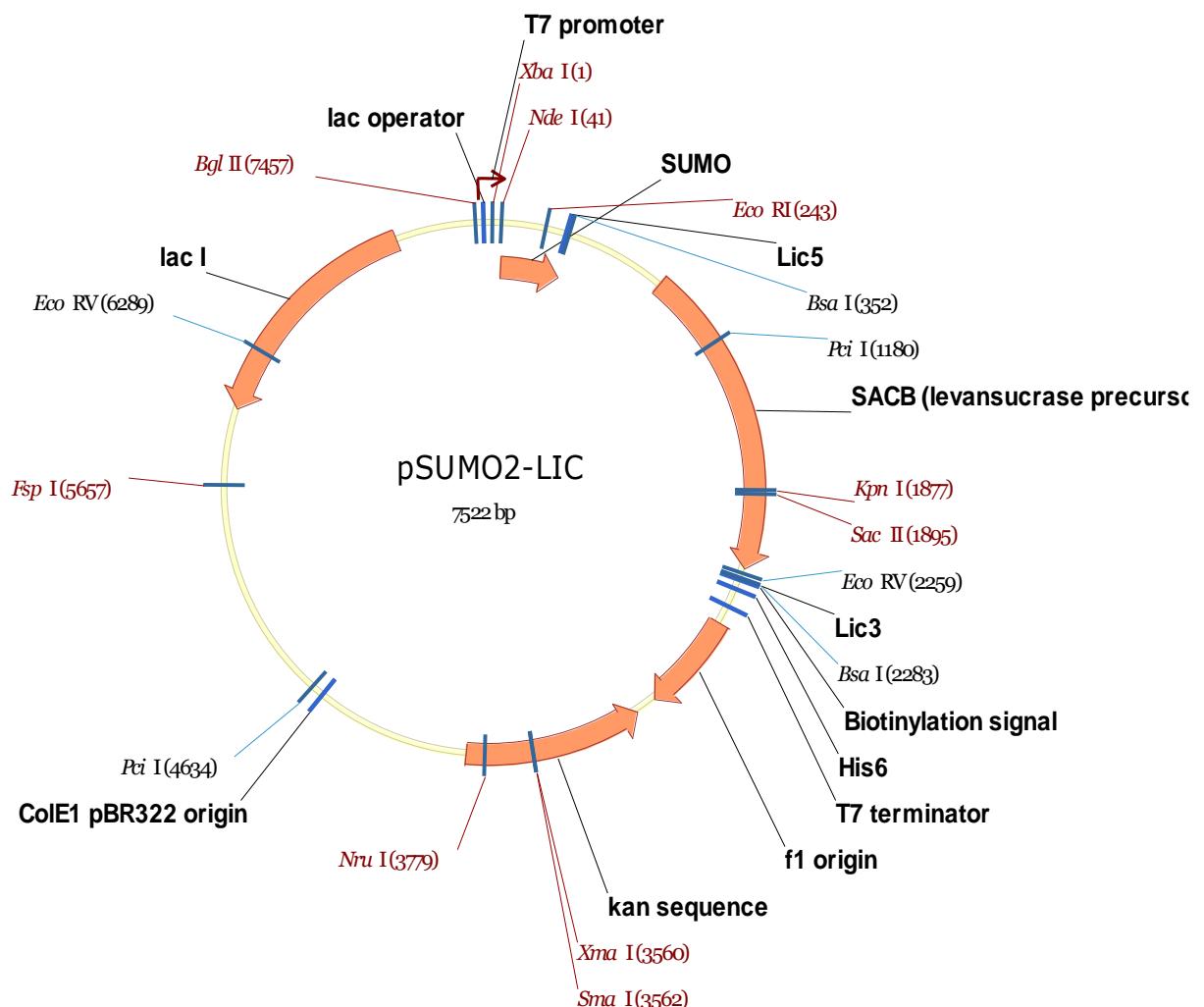
# Vector information sheet

Dated: 16<sup>th</sup> September 2013

Vector Name	<b>pSUMO2-LIC</b>
Source	Pavel Savitsky
Sequence accession/link	Genbank XXXXXXXX

Description	pET expression vector with N-terminal SUMO (96-aa sequence derived from SUMO1 protein) tags followed by a SUMO protease cleavage site and C-terminal biotinylation and His <sub>6</sub> tags. SUMO tag has been shown to improve solubility of the fusion partners. The vector includes sites for LIC cloning, and a “stuffer” fragment that includes the SacB gene, allowing negative selection on 5% sucrose.
-------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dTTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MCSSGSGSGSDQEAKPSTEDL GDKKEGEYIKLK VIGQDSSEI HFKVKMTTHLKKL KESYCQRQGPVMNSLRFLFEGQRIADNHT PKELGMEEEDVIEVYQEQTGG* (* - SUMO cleavage site)
N-terminal fusion – MW	11755.1 Da
C-terminal fusion – seq	SGGGLNDIFEAQKIEWHEHHHHHH
C-terminal fusion – MW	2853 Da
Termination codons	No stop codon
Protease cleavage	SUMO
Additional features	.
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pLIC-for: TGTGAGCGGATAACAATTCC
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC



Cloning region in the vector:

XbaI ~~~~~ NdeI ~~~~~

1 CTAGAAATAA TTTGTTTAA CTTAAGAAG GAGATATACA TATGTGCAGC AGCGGCAGCG

61 · S G S D Q E A K P S T E D L G D K K E G ·  
· GCAGCGGCTC TGACCAGGAG GCAAAACCTT CAACTGAGGA CTTGGGGAT AAGAAGGAAG

121 · E Y I K L K V I G Q D S S E I H F K V K ·  
· GTGAATATAT TAAACTCAAA GTCATTGGAC AGGATAGCAG TGAGATTAC TTCAAAGTGA

181 · M T T H L K K L K E S Y C Q R Q G V P M ·  
· AAATGACAAC ACATCTCAAG AAACTCAAAG AATCATACTG TCAAAGACAG GGTGTTCCA

EcoRI ~~~~~

241 · N S L R F L F E G Q R I A D N H T P K E ·  
· TGAATTCACT CAGGTTTCTC TTTGAGGGTC AGAGAATTGC TGATAATCAT ACTCCAAAAG

Lic5 ~~~~~ BsaI ~~~~~

301 · L G M E E E D V I E V Y Q E Q T G G ·  
· AACTGGGAAT GGAGGAAGAA GATGTGATTG AAGTTTACCA GGAGCAAACG GGAGGTGAGA

BsaI ~~

361 CCGACGTCCA CATATACCTG CCGTTCACTA TTATTAGTG AAATGAGATA TTATGATATT

## SacB

	EcoRV	BsaI
	~~~~~	~~~~~
2221	AACAGTAAAC AAATAAAAAC GCAGGAGAAA ATGCCGATAT CCTATTGGCA TTGACGGTCT	
BsaI		
~		
2281	S G G G L N D I F E A Q K I E W H E H H . CAGCGGTGGC GGTCTGAACG ACATCTCGA GGCTCAGAAA ATCGAATGGC ACGAACACCA	
· H H H H *		
2341	CCACCACCA CACTGAGATC CGGCTGCTAA CAAAGCCCGA AAGGAAGCTG AGTTGGCTGC	

### Lic cloning scheme:

Treat vector with dTTP:

V Y Q E Q T G	G BsaI
<b>GTTT</b> ACCAGGAGCAA <b>ACGG</b>	GAGGT <b>GAGAC</b> GACGTCCACATatacctgcgttactattatttt
CAAATGGTCCTCGTTGCCCTCC	<b>ACTCTGG</b> CTGCAGGTGTAtatggacggcaagtgataataaa

(SacB fragment)

BsaI	S G G G L N D I F E A Q
GATATCCTAT TGGCATTGAC <b>GGTCTCA</b>	GCGGTGGCGGTCTGAACGACATCTCGAGGCTCAG
CTATAGGATA ACCGTAAC TG <b>CCAGAGTCGC</b>	<b>CACCGCCAGAC</b> TTGCTGTAGAAGCTCCGAGTC
K I E W H E H H H H H H	
AAAATCGAATGGCACGAA <b>CA</b> CCACCACCA CACCAC <b>TGAGATCCGGCTGC</b>	
TTTAGCTTACCGTGCTT <b>GT</b> GGTGGTGGTG GTGGTG <b>ACT</b> CTAGGCGACG	

Treat PCR product with dATP:

ACCAGGAGCAA <b>ACGGGAGGT</b>	PCR product	<b>AGCGGTGGCGGTCTG</b>
TGGTCCTCGTTGCCCTCC <b>A</b>		TCGCCACCGCCAGAC

Primers LIC extensions:

Forward	ACCAGGAGCAA <b>ACGGGAGGT</b>
Reverse	CAGACGCCACCGCT

Primers for LIC cloning (treat PCR fragment with T4 DNA polymerase in presence of dATP)

Upstream: add ACCAGGAGCAA**ACGGGAGGT** to the 5' end of upstream primer

Downstream: add CAGACGCCACCGCT to 5' end of downstream primer

```
>pSUMO2-LIC
ctagaataatttgttaactttaaagaaggagatatacatatgtgcagcagcgccagcggcagcggct
ctgaccaggaggcaaaaacctcaactgaggacttggggataagaaggaaaggtaatattaaactca
aagtcatggacaggatagcagttagattcactcaaagtggaaatgacaacacatctcaagaaactca
aagaatcatactgtcaaagacagggtgttccaatgaattcactcagggttctttgagggtcagagaa
ttgctgataatcatactccaaaagaactgggaatggagaaagaagatgtgattgaagttaccaggagc
aaacgggagggtgagaccgacgtccacatatacctgcgttactattattttgtgaaatgagatattat
gatattttctgaattgtgataaaaaggcaactttatgcccattgcaacagaaactataaaaaatacaga
gaatggaaagaaacagatagattttagttctttaggcccgtagtctgcaaatttttatgatttc
tatcaaacaaaagaggaaaatagaccatgtcaatccaaacgagagtctaataagaatgaggtcgaaaag
taaatcgcgcgggttgttactgataaaggcaggcaagcacctaaatgtgtaaaggcaaggtgtataact
ttggcgtcacccttacatatttttaggtttttattgtcgtaactaacttgcacatcttcaaacag
gagggtggaaagaaggcagaccgctaacacagtgatgaaatgagatgtcaacatcaaaa
gtttcaaaaacaagcaacagtattaacctttactaccgcactgctggcaggaggcgcaactcaagcgtt
```



gcgtccagctcggtgatttctccagaaggcgttaatgtctggcttcgataaaagcggccatgttaagg  
gcggtttttcctgtttggactgtatgcgttccgttaaggggatttctgttcatggggtaatgata  
ccgatgaaacgagagaggatgtcacgatacgggtactgtatgatgaaacatgcccggtaactggAACGT  
tgtgagggttaaacaactggcggtatggatgcggcggaccagagaaaaatcaactcagggtcaatgccag  
cgcttcgttaatacagatgttaggtgttccacaggtagccacgcgtatgcgtatgcagatccggAAC  
ataatgtgcaggcgctgacttccgcgtttccagactttacgaaacacggaaaccgaagaccattcat  
gttgggtctcaggcgacgtttgcagcagcgtcgcttcacgtcgctcgatcggtattca  
ttctgctaaccagataaggcaaccccgccagcctagccggctcaacgacaggacacgatcatgcgc  
acccgtggggccggcatgcggcgataatggctgtttctgcggaaacgttggggccggaccagt  
acgaaggcttgagcgaggggcgtcaagattccgaataccgcaagcgcacaggccatcatgcgcgc  
cagcgaaacgcggcctcgccaaaaatgacccagagcgcgtccggcacctgtcctacgagttgcatgata  
aagaagacagtataactgtcgccgcacgatagtcatgcggccacccggaaaggagctgactgggt  
aaggcttcaggcgatcggtcgagatccgggtgcataatgagacttacattaattgcgttgc  
cgctcaactgcccgtttccagtcggaaacctgtcgccagcgtcattatgaaatcgcccaacgcgc  
gggagaggcggttgcgtattggcgccagggtgggttttttaccagtgagacggcaacagct  
attgccttcaccgcgtggccctgagagagttgcagcaagcggtccacgcgtgttgcggccaggcg  
aaaatctgtttgatgggttaacggcggtataacatgagactgtctcggtatcgatccac  
taccgagatatccgcaccaacgcgcagccggactcggtaatggcgccattgcgcggccatctg  
atcggtggcaaccagcatcgccgtggaaacgtgcgtccattcgcatttgcgtgttgcgg  
ggacatggcactcgcgtccgttccgcgtatcggtgttgcgttgcgttgcgg  
ccagccagccagacgcgcacgcgcggagacagaacttaatggccgcataacgcgcgttgcgt  
acccaatgcgaccagatgcgtccacgcgcgttgcgttgcgttgcgttgcgg  
gggtgtctggtcagagacatcaagaataacgcggaaacattagtgccaggcagctccacagcaatggc  
atccctgtcatccagcgatgttaatgatcgcgttgcgttgcgttgcaccgc  
cgcttacaggcttcgcgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
gcgagatataatgcgcgcacaatttgcgcgttgcgttgcgttgcgttgcgttgcgttgc  
cagcaacgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
cgcttcactttccgcgtttcgagaaacgtggctggctggttcaccacgcggaaacggct  
ataagagacaccggcatacttcgcgttgcgttgcgttgcgttgcgttgcgttgc  
actcttcggcgctatcatgcgcatttgcgttgcgttgcgttgcgttgcgttgc  
gacgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ccgcccgaaggatggtcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
tacccacgcgcgaacacaaggcgctatgcgttgcgttgcgttgcgttgcgttgc  
cgatataaggcgccagcaaccgcacctgtggcgccgtatgcgttgcgttgcgttgc  
tcgagatctcgatccgcgaattataactcgactcactataggaaattgtgagcggataacaattcccc  
t

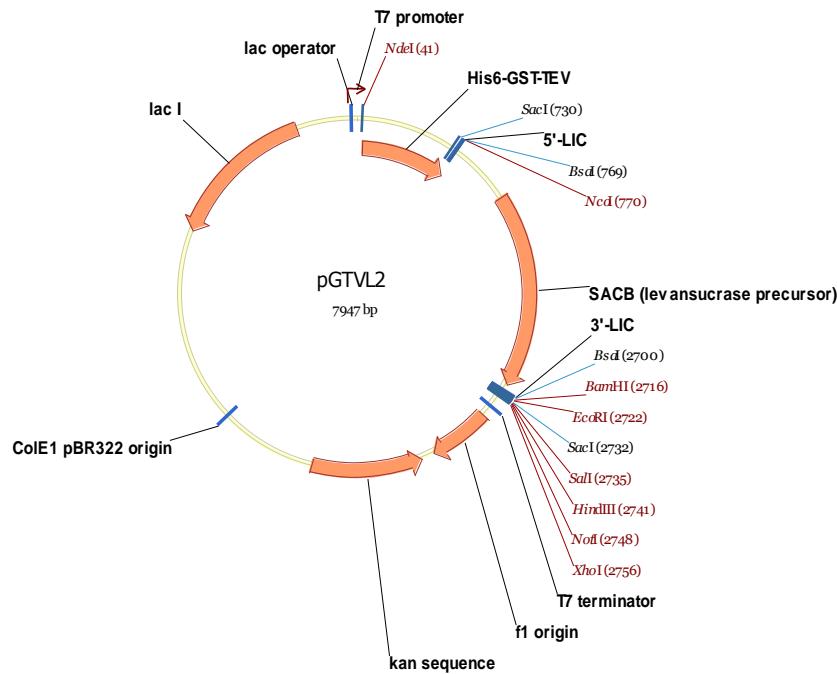
# Vector information sheet

Dated: 8<sup>th</sup> May 2013

Vector Name	<b>pGTVL2</b>
Source	Pavel Savitsky
Sequence accession/link	

Description	pET expression vector with His <sub>6</sub> and GST tag at the N-terminal fusion, followed by TEV protease cleavage site. Includes sites for LIC cloning, and a "stuffer" fragment that includes the SacB gene, allowing negative selection on 5% sucrose
-------------	---

Antibiotic resistance	Kanamycin, 50 µg/ml
Promoter	T7 - lacO
Cloning	LIC. (vector treated with Bsal, then with T4 DNA polymerase in presence of dGTP)
Initiation codon	Supplied in PCR primer
N-terminal fusion – seq.	MHHHHHHSSMSPILGWIKGLVQPTRLLLEYLEEKYEEHYERDEGD KWRNKKFELGLEFPNLPPYYIDGDKLTQSMAIIRYIADKHNMLGGCPKE RAEISMLEGAVLDIYRGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRL CHKTYLNGDHVTPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIA IPQIDKYLKSSKYIAWPLQGWQATFGGDHPPKSSSGVDLGTEONLYFQ SM  (* - TEV cleavage site)
N-terminal fusion – MW	28441 Da including Met
Termination codons	supplied in PCR primer
Protease cleavage	TEV
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	pGEX-for: ATAGCATGGCCTTGCAGG
3' sequencing primer	pLIC-rev: AGCAGCCAACTCAGCTTCC or T7R



### Tag and Polylinker sequence (SacB fragment not shown):

M H H H H H H .  
 1 CTAGAAATAA TTTGTTAA CTTAAGAAG GAGATATACA TATGCACCAT CATCATCATC  
 GATCTTATT AAAACAAATT GAAATTCTTC CTCTATATGT ATACGTGGTA GTAGTAGTAG  
 S S M S P I L G Y W K I K G L V Q P T R .  
 61 ATTCTCTAT GTCCCTATA CTAGTTATT GGAAATTAA GGGCTTGTG CAACCCACTC  
 TAAGAGATA CAGGGGATAT GATCCAATAA CCTTTAATT CCCGAAACAC GTGGGTGAG  
 L L L E Y L E E H L Y E R D E G .  
 121 GACTCTTT GGAATCTT GAAGAAAAAT ATGAAGAGCA TTTGTATGAG CGCGATGAAG  
 CTGAAGAAA CCTTATAGAA CTTCTTTA TACTTCTCGT AAACATACTC GCGCTACTTC  
 D K W R N K K F E L G L E F P N L P Y Y .  
 181 GTGATAATG GCGAACAAA AAGTTGAAT TGGGTTGGA GTTCCCATT CTTCCATT  
 CACTATTAC CGCTTGTT TTCAACCTA ACCCAAACCT CAAAGGGTA GAAGGAATAA  
 I D G D V K L T Q S M A I I R Y I A D K .  
 241 ATATTGATGG TGATGTTAAA TTAACACAGT CTATGGCCAT CATACTTAT ATAGCTGACA  
 TATAACTAC TATACTATT AATTGTC GATACGGTA GTATGCAATA TATCGACTGT  
 H N M L G G C P K E R A E I S M L E G A .  
 301 AGCACAAACAT GTGGGTGGT TGTCCAAAAG AGCGTGCAGA GATTCATG CTTGAAGGAG  
 TCGTGTGTA CAACCCACCA ACAGGTTTC TCGCACGCT CTAAAGTTAC GAACTCCCTC  
 V L D I R Y G V S R I A Y S K D F E T L .  
 361 CGGTTTGGG TATTAGATAC GGTGTTCGA GAATTGCATA TAGTAAAGAC TTTGAAACTC  
 GCCAAAACCT ATAATCTATG CCACAAAGCT CTTAACGTAT ATCATTCTG AAACTTGAG  
 K V D F L S K L P E M L K M F E D R L C .  
 421 TCAAAGTTGA TTTCTTAGC AAGCTACCTG AAATGCTGAA AATGTTGAA GATCGTTAT  
 AGTTCAACT AAAAGAATCG TTCGATGGAC TTACAGCTT TTACAAGCTT CTAGCAAATA  
 H K T Y L N G D H V T H P D F M L Y D A .  
 481 GTCATAAAAC ATATTTAAAT GGTGATCATG TAACCCATCC TGACTTCATG TTGTATGAGC  
 CAGTATTTG TATAATTAA CCACTAGTAC ATTGGTAGG ACTGAAGTAC AACATACTGC  
 L D V V L Y M D P M C L D A F P K L V C .  
 541 CTCTTGATGT TGTTTATAC ATGGACCAA TGTGCCTGGA TGCCTTCAAAATTAGTTT  
 GAGAACTACA ACAAAATATG TACCTGGTT ACACGGACT ACGCAAGGGT TTTAATCAA  
 F K K R I E A I P Q I D K Y L K S S K Y .  
 601 GTTTAAAAAA ACGTATTGAA GCTATCCCAC AAATTGATAA GTACTTGAAA TCCAGCAAGT  
 CAAAATTCTT TGCATAACTT CGATAGGGTG TTTAACTATT CATGAACCTT AGTCGTTCA  
 pGEX-for  
 -----→  
 I A W P L Q G W Q A T F G G G D H P P K .  
 661 ATATAGCATG GCCTTGCAG GGCTGGCAAG CCACGTTGG TGGTGGCGAC CATCCTCAA  
 TATATCGTAC CGGAAACGTC CGCACCGTTC GGTGCAACC ACCACCGCTG GTAGGAGGT

Lic5 BsaI

721 S S S G V D L G T E N L Y F Q S M ~~~~~  
 AATCGAGCTC AGGTGTTAGAT CTGGGTACCG AGAACCTGT A CTTCCAATCC ATGGAGACCG  
 TTAGCTCGAG TCCACATCTA GACCCATGGC TCTTGGACAT GAAGGTTAGG TACCTCTGGC

118 ----- (SacB fragment) -----  
 -----

2010 EcoRI  
 BsaI LIC 3' BamHI ~~~~~ SacI  
 GATATCCTAT TGGCATTGAC GGTCTCCAGT AAAGGTGGAT ACGGATCCGA ATTGAGCTC  
 CTATAGGATA ACCGTAAC TG CCAGAGGTCA TTTCCACCTA TGCCTAGGCT TAAGCTCGAG

2070 SalI  
 HindIII  
 \*\*\*\*\*~~~~~  
 CGTCGACAAG CTTGCGGCCG CACTCGAGCA CCACCACAC CACCACTGAG ATCCGGCTGC  
 GCAGCTGTC GAACGCGGC GTGAGCTCGT GGTGGTGGTG GTGGTGACTC TAGGCGACG  
 T7-reverse  
 ←-----  
 2130 TAACAAAGCC CGAAAGGAAG CTGAGTTGGC TGCTGCCACC GCTGAGCAAT AACTAGCAT  
 ATTGTTTCGG GCTTTCTTC GACTCAACCG ACGACGGTGG CGACTCGTTA TTGATCGTAT  
 ←-----  
 pLIC-rev

## Primers for LIC cloning:

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

Downstream: add TATCCACCTTACTGtca to 5' end of downstream primer; (the final tca provides a termination codon)

pGTVL2 sequence:

ctgaaaataatttgttaactttaagaaggagatatacatatgcaccatcatcatcatcattttctatgtccctatacttaggttattggaaaattaagggcctgtcaacccactcgacttcttggaaatatcttgaagaaaatatacgagcattgtatgagcgcgatgaaggtgataaatggcggaaacaaaaagtttgaaattgggttggagttcccaatcttcatttatattatgtatggatgttaattaaacacagtctatggccatcatacgttatatacgacaagcacaacatgtgggtgggtccaaaagagcgtgcagagatttcaatgctgaaggagcggggttggatattagatacgggtttcgagaattgcataatagtaaagactttgaaactctcaaagttgatttcttagcaagctacctgaaaatgttgcagatcgtttagtgcataaaaacatatttaatgggtatcatgttaaccatcctgacttcatgttgcacgccttgcattgttgcatttatacatggacccaatgtgcctggatgcgttccaaaattagttttttaaaaaacgtattgaagctatcccacaaattgataagtacttgaatccagcaagtatatacgatggccttgcagggctggcaagccacggttgggtggcggaccatcctccaaaatcgagctcaggtgttagatctgggtaccggagaacctgtacttccaaatccatggagaccgacgtccacatatacctgcgcgttcaactattatttagtggaaatgagatattatgtatatttctgaattgtgattaaaaggcaactttatgcccattgcacacagaaactataaaaaatacagagaatgaaaagaaaacagatagatttttagttcttaggcggtagtctgcacaaatcctttatgatttctatcaaacaaaagaggaaaatagaccagtgcacatccaaacgagagtctaataatgaggtcgaaaggtaaatcgccgggttgcattgtgataaaagcaggcaagcacactaaatgtgtaaaggcggaaagtgtatacttggcgtcaccccttacatatttttaggtctttttattgtgcgtactaacttgcacatcttcaaaaggaggcgtggaaagaaagcagaccgctaaacacagttacataaaaaaggagacatgaacgatgaacatcaaaggatttgcacaaacagcaacagttacccattaccgcactgtgcggcaggaggcgaactcaagcgttgcggaaagaaaacgaccaacccatataaggaaacatacggcatttccatattacacgcgttgcattgtcaatccctgtatgcacatcttgcacacagaaaaatataaagttcctgagttcgattcgatcgttccacaataaaaatatcttgcacaaaggcctggacgttggacagctggccattacaaaacactgacggcactgtgcggcaactatcgttgcacactatcggctaccacatcgtcttgcattagccggagatcctaaaaatgcggatgacacatcgattacatgttctataaaaagtcggcggaaacttctattgcacagctggaaaaacgcgtggccgcgtctttaagacagcggacaaattcgatgcacaaatgattctatcctaaaagaccaacacaagaatggtcagg

ttcagccacattacatctgacggaaaatccgttattctacactgattctccgtaaacattacgg  
caaacaacactgacaactgcacaagttaacgtatcagcatcagacagctcttgaacatcaacggtgt  
agaggattataaatcaatcttgacggtgacggaaaacgtatcaaaatgtacagcagttcatcgatga  
aggcaactacagtcagggcacaaccatacgtgagagatcctcaactacgtagaagataaaggccacaa  
atacttagtatttgaagcaaactgaaactgaaatggctaccaaggcagaagatcttatttaacaa  
agcatactatggcaaaagcacatcattctccgtcaagaaagtcaaaaacttctgcaaagcgataaaaa  
acgcacggctgagtttagcaaacggcgctcggatgattgagctaaacacgtattacacactgaaaaa  
agtatgaaaccgctgattgcatctaaccacagtaacagatgaaattgaacgcgcgaacgtctttaaaat  
gaacggcaatggtacctgtcactgactcccggatcaaaaatgacgattgacggcattacgtctaa  
cgatattacatgcttggtatgtttaattcttaactggccatacaagccgctgaacaaaactgg  
ccttggtttaaaatggatottgatctaaccatgatgtaccccttacttactcacacttcgctgtacctca  
agcgaaaggaaacaatgtcgtgattacaagctatatgacaacacagaggattctacgcagacaaacaatc  
aacgttgcgcctagcttcgtctgaacatcaaaggcaagaaaacatctgttgcattaaagacacgcattc  
tgaacaaggacaattaacagtaacaataaaaacgcggaaaagaaaatgccatatttgcattga  
cggtctccagtaaagggtggatccgatccgaaattcgagctccgtcgacaagcttgcggccgactcgag  
caccaccaccaccactgagatccggctgctaacaaagccccaaaggaagctgagttggcgtgtgcc  
accgctgagcaataactagcataaccctggggctctaaacgggtcttgcagggtttttgtgaaa  
ggaggaactatccggattggcgaatgggacgcgcggctgtagcggcgcattaaagcgcggcggtgtgg  
tggttacgcgcagcgtgaccgctacacttgcagcggccctagcgcggcttcgcattttgcatttccctt  
ccttcgcacgttgcggcttccggctcaagctctaattcggggctccctttagggttccgat  
ttagtgcattacggcacccctcgaccccaaaaaacttgattagggtatggtcacgttagtggacttcc  
cctgatagacggttttcgccttgcacgttgcggatccgatctttaatagtggacttgcatttcc  
ctggaaacaacactcaaccctatctggcttattttgattataagggatttgcgatttcggcct  
attggtaaaaaatgagctgatthaacaaaatttaacgcgatattaaatattaacgttacaa  
tttcaggtggcactttcgggaaatgtgcgcggaaaccctattgttattttctaaatacattcaa  
atatgtatccgctcatgatthaattcttagaaaactcatcgagcatcaaactgcaatttattc  
atatcaggattataccatattttgaaaaagccgttctgtaatgaaggagaaaactcaccgagg  
cagttccataggatggcaagatcctggatcggtctgcgattccgactcgccaaatcaatacc  
attaatttccctcgtaaaaataagggtatcaagtgagaaatcaccatgagtgacgactgaatccgg  
gagaatggcaaaagttatgcatttccagacttgcattaaacaggccaggccattacgctcgatca  
aaatactcgcatcaaccaaccgttattcattcggtattgcgcctgagcgagacgaaatcgcgatcg  
ctgttaaaaggacaattacaaaacaggaatcgaatgcacccggcaggaaacactgcccgcattcaaca  
atatttcacctgaatcaggatattctcttaataacctggatgtctgtttccgggatcgcatgg  
agtaaccatgcatcatcaggagtagcgataaaatgttgcattttccggatggccatgcgc  
cagtttagtgcatttcatctgttaacatcatgttgcacgttgcattttccggatggccatgc  
tctggcgcattccatcggatggccatcggatggccatgcgcattttccggatggccatgc  
catttataccatataatcagcatcatgttgcattttccggatggccatgcgcattttccgg  
tgaatatggctcataacacccttgcattactgttgcattttccggatggccatgc  
aatcccttaacgtgagtttgcgttccactgagcgtcagaccggtagaaaagatcaaggatcttctt  
agatcccttttgcgttaatctgtgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
tttgcggatcaagagacttccggatggccatgcgttgcgttgcgttgcgttgcgttgc  
tactgtccttcttagtgcgttagtgcgttagtgcgttgcgttgcgttgcgttgc  
cgctctgtaatctgttaccagggttgcgttgcgttgcgttgcgttgcgttgcgttgc  
aagacgatagttaccggataaggcgcagcggctggctgaacgggggttgcgtc  
ggagcgaacgcgttaccggatggccatgcgttgcgttgcgttgcgttgcgttgc  
aggggaaaaggcgttaccggatggccatgcgttgcgttgcgttgcgttgcgttgc  
agggggaaaacgcgttaccggatggccatgcgttgcgttgcgttgcgttgcgttgc  
gtatgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ctttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
cgcttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
agcggaaagagcgcctgtatggccatgcgttgcgttgcgttgcgttgcgttgc  
tgcactctcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
actgggtcatggcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
cgcatccgttacagacaagctgtgaccgtctccggagctgcattgtgtc  
caccgaaacgcgcggaggcagctgcgttgcgttgcgttgcgttgcgttgc  
cctgttcatccggcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
ccatgttacggccatgcgttgcgttgcgttgcgttgcgttgcgttgc  
ggtaatgataccgatgaaacgcggatggccatgcgttgcgttgcgttgc  
tactggaaacgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
gtcaatgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgc  
agatccggaaacataatggcgttgcgttgcgttgcgttgcgttgcgttgc

agaccattcatgttgttgcctaggcgcagacgtttgcagcagcagtcgtcacgttcgctcgcta  
tcgggtattcattctgctaaccaggtaaggcaaccccgccagcctagccgggtcctcaacgacaggagca  
cgatcatgcgcacccgtggggccatgccggcgataatggcctgcttcgcggaaacgttgg  
cgggaccagtgaagaaggcttgagcgagggcggtgcaagattccgaataccgcaagcgacaggccatca  
tcgtcgcgctccagcgaagcggtcctcgccgaaaatgaccagagcgctgccgcacctgtcctacga  
gttgcataaagaagacagtataaagtgcggcgacgatagtcatgcggcgccaccggagg  
tgactgggttgaaggctctaaggcatcggtcgagatcccgtgcctaattagtgagactaattacat  
taattgcgttgcgcgactgcggcgttccagtcggaaacctgtcgccagctgcattaatgaatcg  
gccaacgcgcggggagaggcggttgcgtattggcgccagggtggtttttaccagtgagacg  
ggcaacagctgattgcgccttcaccgcctggccctgagagagttgcagcaagcggtccacgcgg  
cccgaggcgaaaatcctgtttgatggtgtaacggcgggatataacatgagctgttgcgtatcg  
tcgtatcccactaccgagatatccgcaccaacgcgcagccccgactcggtaatggcgccattgcgc  
agcgccatctgatcggtggcaaccagcatcgcaigtggaaacgatgcctcattcagcattgcatt  
tggtaaaaccggacatggcactccagtcgcctccgttccgcatacgctgaaatttgcgact  
agatatttatgcgcagccagccagacgcagacgcggcggagacagaacttaatggccgc  
attingctggtgacccaatgcgaccagatgcgtccacgcggcgttgcgttgcattgcgc  
atactgttgcgtgggtctggcagagacatcaagaataacgcggaaacattgcaggc  
acagcaatggcattctggcatccagcgatagttaatgatcagccactgcgcgttgcgc  
ttgtgcaccgcgccttacaggcttcgacgcgcgttcgttgcattaccatgc  
agttgatcgccgcgagatttaatgcgcgcacaatttgcgcgcgtgcagg  
gcaacgccaatcagcaacgactgttgcgcgcgttgcgcgcgttgc  
tccgcattgcgcgcgttccactttccgcgtttcgcagaaaacgtgg  
gaaacggctgataagagacaccggcatactctgcgcacatcg  
accctgaattgactcttcgcgtatcatgcgcataccgc  
tccggatctcgacgcgtcccttatgcgcactccgcatt  
gttgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
gcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgcgc  
ggcgttagaggatcgagatctcgatccgc  
aacaattcccc