

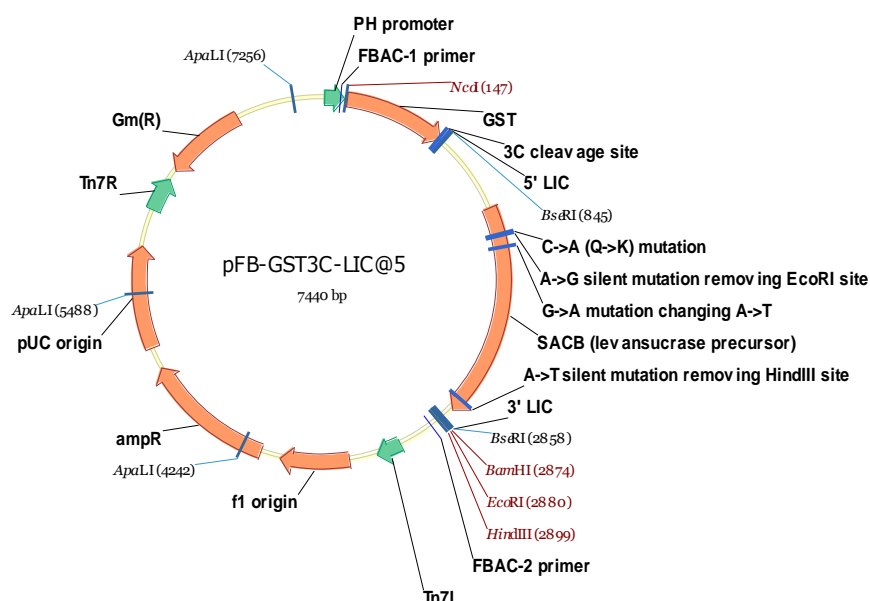
Vector information sheet

Dated: 27^h January 2015

Vector Name	pFB-GST3C-LIC
Source	Claire Strain-Damerell
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
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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.	MGSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEF PNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYG VSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYD ALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLEVLQ*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: TATTCATACCGTCCCACCA
3' sequencing primer	FBAC2: GGGAGGTTTTTTAAAGCAAGTAAA



Cloning region in the vector:

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                                NcoI
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                                M   G   S   P   I   L   G   Y   W   K   I
121  TCGGGCGCGG ATCTCGGTCC GAAAACCATG GGCTCCCCTA TACTAGGTTA TTGGAAAATT
    AGCCCGCGCC TAGAGCCAGG CTTTGGGTAC CCGAGGGGAT ATGATCCAAT AACCTTTTAA
    K   G   L   V   Q   P   T   R   L   L   L   E   Y   L   E   E   K   Y   E   E
181  AAGGGCCTTG TGCAACCCAC TCGACTTCTT TTGGAATATC TTGAAGAAAA ATATGAAGAG
    TTCCCGGAAC ACGTTGGGTG AGCTGAAGAA AACCTTATAG AACTTCTTTT TATACTTCTC
    H   L   Y   E   R   D   E   G   D   K   W   R   N   K   K   F   E   L   G   L
241  CATTTGTATG AGCGCGATGA AGGTGATAAA TGGCGAAACA AAAAGTTTGA ATTGGGTTTG
    GTAAACATAC TCGCGCTACT TCCACTATTT ACCGCTTTGT TTTTCAAAC TAAACCAAAC
    E   F   P   N   L   P   Y   Y   I   D   G   D   V   K   L   T   Q   S   M   A
301  GAGTTTCCCA ATCTTCCTTA TTATATTGAT GGTGATGTGA AATTAACACA GTCTATGGCC
    CTCAAAGGGT TAGAAGGAAT AATATAACTA CCACTACAAT TTAATTGTGT CAGATACCGG
    I   I   R   Y   I   A   D   K   H   N   M   L   G   G   C   P   K   E   R   A
361  ATCATACGTT ATATAGCTGA CAAGCACAAC ATGTTGGGTG GTTGTCCTAA AGAGCGTGCA
    TAGTATGCAA TATATCGAGT GTTCGTGTTG TACAACCCAC CAACAGGTTT TCTCGCACGT
    E   I   S   M   L   E   G   A   V   L   D   I   R   Y   G   V   S   R   I   A
421  GAGATTTCAA TGCTGAAGG AGCGGTTTTG GATATTAGAT ACGGTGTTTC GAGAATTGCA
    CTCTAAAGTT ACGAACTTCC TCGCCAAAAC CTATAATCTA TGCCACAAAG CTCTTAACGT
    Y   S   K   D   F   E   T   L   K   V   D   F   L   S   K   L   P   E   M   L
481  TATAGTAAAG ACTTTGAAC TCTCAAAGTT GATTTTCTTA GCAAGCTACC TGAAATGCTG
    ATATCATTTC TGAAACTTTG AGAGTTTCAA CTAAAAGAAT CGTTCGATGG ACTTTACGAC
    K   M   F   E   D   R   L   C   H   K   T   Y   L   N   G   D   H   V   T   H
541  AAAATGTTTC AAGATCGTTT ATGTCATAAA ACATATTTAA ATGGTGATCA TGTAACCCAT
    TTTTACAAGC TTCTAGCAAA TACAGTATTT TGTATAAATT TACCACTAGT ACATTGGGTA
    P   D   F   M   L   Y   D   A   L   D   V   V   L   Y   M   D   P   M   C   L
601  CCTGACTTCA TGTGTATGA CGCTCTTGAT GTTGTTTTAT ACATGGACCC 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  GATGCGTTCC CAAAATTAGT TTGTTTTAAA AAACGTATTG AAGCTATCCC ACAAATTGAT
    CTACGCAAGG GTTTTAATCA AACAAAATTT TTTGCATAAC TTCGATAGGG TGTTTAACTA
    K   Y   L   K   S   S   K   Y   I   A   W   P   L   Q   G   W   Q   A   T   F
721  AAGTACTTGA AATCCAGCAA GTATATAGCA TGGCCTTTGC AGGGCTGGCA AGCCACGTTT
    TTCATGAAC TTAGGTCGTT CATATATCGT ACCGGAACG 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 CTGGAAGTTC TGTTCAGGG CCCACTCTCT
    CCACCACCGC TGGTAGGAGG TTTTAGCCTA GACCTTCAAG ACAAGGTCCC GGGTGAGAGA
                                BseRI
                                ~~~~~~
841  ATCCGCTAGC TTCTCCTCCT GAAAGATCCA TAACTTCGTA TAGCATACAT TATACGAAGT
    TAGGCGATCG AAGAGGAGGA CTTTCTAGGT ATTGAAGCAT ATCGTATGTA ATATGCTTCA

----- SacB -----

                                BseRI                                BamHI
                                ~~~~~~                                ~~~~~~
2821 TGACGTCAGG TGGCACTTTT CGAGGAGTTT ACTAGTAAGT AAAGGTGGAT ACGGATCCGA
    ACTGCAGTCC ACCGTGAAAA GCTCCTCAAA TGATCATTCA TTTCCACCTA TGCTTAGGCT

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Primers for LIC cloning:

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

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