

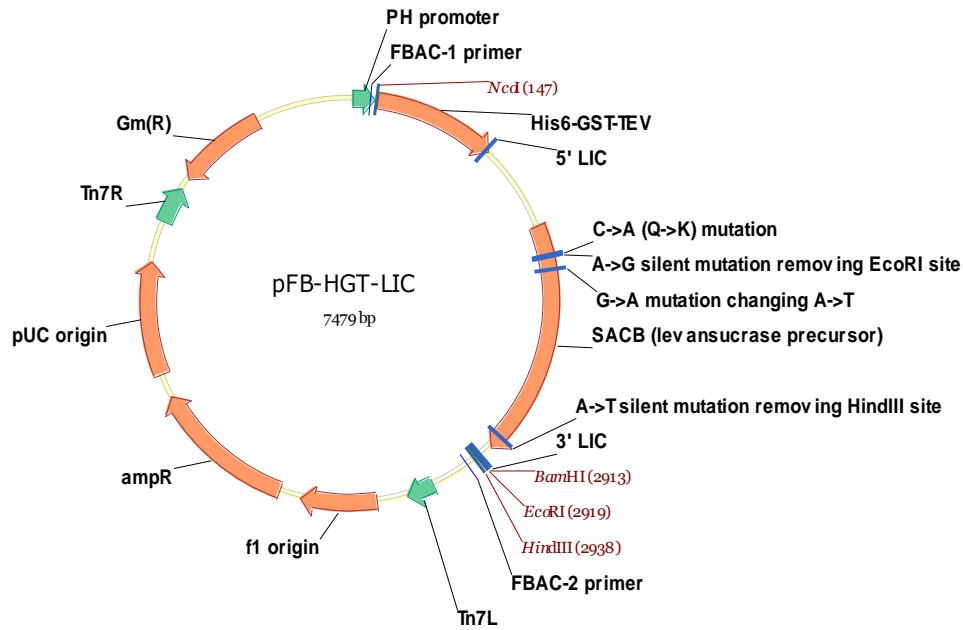
Vector information sheet

Dated: 8th May 2013

| | |
|-------------------------|----------------------|
| Vector Name | pFB-HGT-LIC |
| Source | Nicola Burgess-Brown |
| Sequence accession/link | (SGC) |

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|-------------|--|
| Description | Baculovirus transfer vector with His ₆ + GST tags in 243-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 |
|-------------|--|

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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. | MGHHHHHHSSMSPILGYWKIKGLVQPTRLLEYLEEKYEEHLY ERDEGDKWRNKKFELGLEFPNLPYYIDGDVCLTQSMIIIRYIAD KHNMLGGCPKERAEISMLEGAVLDIRYGVSR IAYSKDFETLKVD FLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLY MDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQGWQA T FGGGDHPPKSSSGVDLG TENLYFQ*SM (* - TEV cleavage site) |
| N-terminal fusion – MW | 28280 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: GGGAGGTTTTTTAAAGCAAGTAAA |



NcoI
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121      M G H H H H H H S S M
      ATG GGCCACCATC ATCATCATCA TTCTTCTATG
      TAC CCGGTGGTAG TAGTAGTAGT AAGAAGATAC
181      S P I L G Y W K I K G L V Q P T R L L L
      TCCCCTATAC TAGGTTATG GAAAATTAAG GGCCTTGTGC AACCCACTCG ACTTCTTTTG
      AGGGGATATG ATCCAATAAC CTTTAAATTC CCGGAACACG TTGGGTGAGC TGAAGAAAAC
      E Y L E E K Y E E H L Y E R D E G D K W
241      GAATATCTTG AAGAAAAATA TGAAGAGCAT TTGTATGAGC GCGATGAAGG TGATAAATGG
      CTTATAGAAC TTCTTTTTAT ACTTCTCGTA AACATACTCG CGCTACTTCC ACTATTTACC
      R N K K F E L G L E F P N L P Y Y I D G
301      CGAAACAAA AGTTGAATT GGGTTTGAG TTTCCCAATC TTCCTTATTA TATTGATGGT
      GCTTTGTTTT TCAAACCTAA CCCAAACCTC AAAGGGTTAG AAGGAATAAT ATAACTACCA
      D V K L T Q S M A I I R Y I A D K H N M
361      GATGTTAAAT TAACACAGTC TATGGCCATC ATACGTTATA TAGCTGACAA GCACAACATG
      CTACAATTTA ATTGTGTCAG ATACCGGTAG TATGCAATAT ATCGACTGTT CGTGTGTGATC
      L G G C P K E R A E I S M L E G A V L D
421      TTGGGTGGTT GTCCAAAAGA GCGTGCAGAG ATTTCAATGC TTGAAGGAGC GGTTTTGGAT
      AACCCACCAA CAGGTTTTCT CGCACGTCTC TAAAGTTACG AACTTCTCTG CCAAACCTA
      I R Y G V S R I A Y S K D F E T L K V D
481      ATTAGATACG GTGTTTCGAG AATTGCATAT AGTAAAGACT TTGAAACTCT CAAAGTTGAT
      TAATCTATGC CACAAAGCTC TTAACGTATA TCATTTCTGA AACTTTGAGA GTTTCAACTA
      F L S K L P E M L K M F E D R L C H K T
541      TTTCTTAGCA AGCTACCTGA AATGCTGAAA ATGTTCGAAG ATCGTTTATG TCATAAAACA
      AAAGAATCGT TCGATGGACT TTACGACTTT TACAAGCTTC TAGCAAATAC AGTATTTTGT
      Y L N G D H V T H P D F M L Y D A L D V
601      TATTTAAATG GTGATCATGT AACCCATCCT GACTTCATGT TGTATGACGC TCTTGTATGTT
      ATAAATTTAC CACTAGTACA TTGGGTAGGA CTGAAGTACA ACATACTGCG AGAACTACAA
      V L Y M D P M C L D A F P K L V C F K K
661      GTTTTATACA TGGACCCAAT GTGCCTGGAT GCGTTCCCAA AATTAGTTTG TTTTAAAAAA
      CAAAATATGT ACCTGGGTTA CACGGACCTA CGCAAGGGTT TTAATCAAAC AAAATTTTTT
      R I E A I P Q I D K Y L K S S K Y I A W
721      CGTATGAAG CTATCCACA AATTGATAAG TACTTGAAS CCAGCAAGTA TATAGCATGG
      GCATAACTTC GATAGGGTGT TTAACTATTC ATGAACTTA GGTGTTTCAT ATATCGTACC
      P L Q G W Q A T F G G G D H P P K S S S
781      CCTTTGAGG GCTGGCAAGC CACGTTTGGT GGTGGCGACC ATCCTCCAAA ATCGAGCTCA
      GGAACGTCG CGACCGTTCG GTGCAAACCA CCACCGCTGG TAGGAGGTTT TAGCTCGAGT
      G V D L G T E N L Y F Q
841      GGTGTAGATC TGGGTACCGA GAACCTGTAC TTCCAATCCA TAAGCTAGCT TCTCCTCTTG
      CCACATCTAG ACCCATGGCT CTTGGACATG AAGGTTAGGT ATTCGATCGA AGAGGAGGAC
901      AAA
      TTT SacB

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2821                                     ACTTTTC
                                           TGAAAAG

                                           EcoRI
                                           ~~~~~~

 BamHI
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                                           HindIII
                                           ~~~~~~

2881 GAGGAGTTTA CTAGTAAGTA AAGGTGGATA CGGATCCGAA TTCGAGCTCC GTCGACAAGC
 CTCCTCAAAT GATCATTGAT TTCCACCTAT GCCTAGGCTT AAGCTCGAGG CAGCTGTTTC
 HindIII
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2941 TTGTCGAGAA
 AACAGCTCTT

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**Primers for LIC cloning:**

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

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

**pFB-HGT-LIC sequence:**

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