Vector information sheet

Dated: Apr 2020

Vector Name	pBacMam2-SDiEx-LIC
Source	Designed by Alma Seitova, constructed by Yanjun Li
Company	Structural Genomics Consortium, Toronto
Description	Baculovirus transfer vector derivative of pBacMam2-DiEx-LIC (Pravin Mahajan, SGC Oxford), with a N-terminal Honeybee melittin signal Tag for expression of secreted proteins in insect as well as in mammalian cells, and C-terminal fusion His10 and FLAG tag preceded by a TEV protease cleavage site. The vector includes a “stuffer” SacB gene flanked by two BfuAI sites for LIC cloning.
Antibiotic resistance	Ampicillin, 100 ug/ml
Promoter	CMV and P10
Cloning	Insertion of DNA sequence into the cloning/expression region is performed using Infusion enzyme mediated directional recombination between 15 complementary nucleotide DNA sequences at the end of the insert PCR and BfuAI linearized vector. Positive clones will be chosen through negative selection procedure, gene of interest replacement of a SacB stuffer sequence on 5% sucrose LB agar plate.
Initiation codon	ATG codon in Honeybee melittin signal sequence
N-terminal Honeybee melittin signal (HBMSS) AA sequence	MKFLVNVALVFMVVYISYIYAAA
C-terminal fusion sequence	AENLYFQSHHHHHHHHHHDYKDDDDK
Termination codon	TGA downstream of Flag tag
Protease cleavage	TEV protease sites enable removal of C-terminal fusion tag yielding a native protein
Additional features	Tn7 sequences for site-specific transposition Into DH10Bac E. Coli competent cells
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’ primer addition	atctatgcggccgct
3’ primer addition	gattggaagtagaggttctctgc
5’ sequencing primer	pFBM-F: caaaatgtcgtaacaactccgc
3’ sequencing primer	pFBSM-R: aaggaggagaaaatgaaagcc

pBacMam2-SDiEx-LIC cloning/expression region:

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                                  Honeybee melittin signal sequence
                                    ~~~~~~~~~~~~~~~~~~~~~~~~
5049 ACAATCACTC GACGGATCCT TAAGAAGGAG ATATACTATG AAATTCTTAG TCAACGTTGC
     TGTTAGTGAG CTGCCTAGGA ATTCTTCCTC TATATGATAC TTTAAGAATC AGTTGCAACG

     Honeybee melittin signal sequence (5086-5154)
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~      BfuAI
                                                               ~~~~~~

5109 CCTTGTTTTT ATGGTCGTAT ACATTTCTTA CATCTATGCG GCCGCTTATA CTATGCAGGT
     GGAACAAAAA TACCAGCATA TGTAAAGAAT GTAGATACGC CGGCGAATAT GATACGTCCA

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

                                                        TEV
                                                  ~~~~~~~~~~~~~~~~~~~~~
                                           BfuAI
                                           ~~~~~~
                                                    A   E  N  L   Y  F  Q  .
7040 GCCGATATCC TATTGGCATT GACGTCAGGT GGCACACCTG CAGAGAACCT CTACTTCCAA
     CGGCTATAGG ATAACCGTAA CTGCAGTCCA CCGTGTGGAC GTCTCTTGGA GATGAAGGTT

                10 His
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~   Flag
                                             ~~~~~~~~~~~~~~~~~~~~~~~~
                                                                        Stop codon
                                                                        ~~~
      S  H  H   H  H  H  H   H  H  H   H  D  Y  K   D  D  D   D  K
7100  TCGCACCATC ATCACCATCA CCATCACCAC CATGATTACA AGGATGACGA CGATAAGTGA
      ACGCAGGATG ATGTGGTAGT GGTAGTGGTG GTACTAATGT TCCTACTGCT GCTATTCACT
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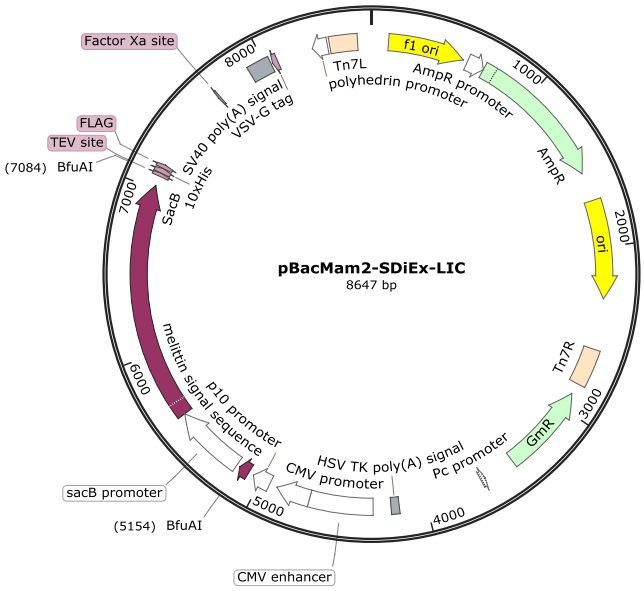
pBacMam2-SDiEx-LIC Vector Map



Full sequence of pBacMam2-SDiEx-LIC:

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