pSFV4 Vector

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pSFV4 vector, aka Super FAB expression vector Version 4, was derived from expression vector pCW-LIC (GenBank accession EF460848). It is used for tac promoter driven expression of FABs with a heavy chain C-terminal biotinylation tag. The vector contains two additional FAB heavy chain sequence additions for adding HIS tag, or adding no tag. These are accessible by RE digestion and ligation within the vector. The vector includes a PelB leader for the Light chain and the constant domain coding sequence of the heavy chain. The vector is intended for receiving FAB (LC and HC) coding sequences from phage display vectors for conversion to bicystronic IPTG inducible expression.

Antibiotic resistance	Ampicillin, 100 ug/ml
Promoter	Three sequential tac promoters, the 2nd two are active
Cloning Methods	Insertion of DNA sequence into the cloning/expression region is preformed using BD-Biosciences Infusion enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and Sph1 linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on LB agar supplemented with 5% sucrose and Ampicillin.
Initiation Codon	NdeI site in vector at 4796 bp
N – terminal sequence included in vector. PelB and FAB LC scaffold.	MKSLLPTAAAGLLLLAAQPAMASDIQMTQSPSSLSASVGDR VTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPS RFSGSRSGTDFTLTISSLQPEDFATYY
C-terminal fusion sequences. HC scaffold and HC tags included in vector	Avi-tag, default: LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTGGSGSAGGLNDI FEAQKIEWHE** No tag, after Sal1 digest and internal vector ligation: LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHT** His-tag, after Sal1/Bsa1 digest and internal vector ligation: LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCDKTHTGGSHHHHHH**

Termination codons	TGATGA or TAATAA included in vector
Preferred Hosts	BL21 strains carrying pBirAcm, an engineered pACYC184 plasmid with an IPTG inducible birA gene to over-express the biotin ligase. If not using Avitag just use a BL21 strain.
5' primer for amplification of FAB LC and HC from Library E or F 3' primer for amplification of FAB LC and HC from Library E or F	pSFV4-FwdClone 5'- cgcaacttattactgtcagc-3' pSFV4-RevClone 5'- agacggtgaccagggttcc-3'
To remove LC flag tag amplify LC and HC separately.	IP-HC-Fwd 5'-gagagtgttaattaactcgaggctgagc-3' pair with pSFV4-RevClone IP-LC- Rev 5'-gttaattaacactctcccctgttgaag-3' Pair with pSFV4-FwdClone
5' sequencing and colony screening primer	pSFV4-SeqV1 5' – tctactctggagtcccttctc- 3'
3' sequencing and colony screening primer	pCW-Rev 5' – ctttcgtcttcaagcagatctg - 3'
Primers for screening colonies after switching	pSFV4ConvSF acaagcccagcaacaccaag pair with pCW-Rev

Notes for Cloning:

1. PCR products can only be inserted into this vector with Infusion enzyme, not via T4 ligase.
2. Perform PCR with PFU ultra II which has a low error rate. PAGE purified primers will also lower cloning error rate.
3. Screen colonies directly by PCR using Taq pol and recommended primers
4. To switch constructs from Avi-tag to no HC tag, digest constructs with Sal1and ligate. This ligation is very efficient and only a small amount of plasmid DNA is needed.
5. To switch constructs from Avi-tag to no Hexa-HIS tag, digest constructs with Sal1 and Bsa1 and ligate. This ligation is very efficient and only a small amount of plasmid DNA is needed.
6. If phagmid has LC flag tag it can be removed by amplification of LC and HC in separate reactions. Then perform a 3 piece infusion reaction with LC, HC and Vector. This reaction is slightly less efficient than a 2 piece reaction.

pSFV4 cloning/expression region

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4685  actccccatc cccctgttga caattaatca tcggctcgta taatgtgtgg
      tgaggggtag ggggacaact gttaattagt agccgagcat attacacacc

                                          BamHI
      aattgtgagc ggataacaat ttcacacagg aaacaggatc catcgatgct
      ttaacactcg cctattgtta aagtgtgtcc tttgtcctag gtagctacga


               NdeI
                  M  K  S   L  L  P   T  A  A  A   G  L  L
      taggaggtcat atgaaatcc ctattgccta cggcagccgc tggattgtta
      atcctccagta tactttagg gataacggat gccgtcggcg acctaacaat

                           Nco1
       L  L  A   A  Q  P  A   M  A  S   D  I  Q   M  T  Q  S
      ttactcgcgg cccagccggc catggcgtcc gatatccaga tgacccagtc
      aatgagcgcc gggtcggccg gtaccgcagg ctctcggtct actgggtcag

        P  S  S   L  S  A   S  V  G  D   R  V  T   I  T  C
      cccgagctcc ctgtccgcct ctgtgggcga tagggtcacc atcacctgcc
      gggctcgagg gacaggcgga gacacccgct atcccagagg tagtggacgg

      R  A  S  Q   S  V  S   S  A  V   A  W  Y  Q   Q  K  P
      gtgccagtca gtccgtgtcc agcgctgtag cctggtatca acagaaacca
      cacggtcagt caggcacagg tcgcgacatc ggaccatagt tgtctttggt

       G  K  A   P  K  L  L   I  Y  S   A  S  S   L  Y  S  G
      ggaaaagctc cgaagcttct gatttactcg gcatccagcc tctactctgg
      ccttttcgag gcttcgaaga ctaaatgagc cgtaggtcgg agatgagacc

        V  P  S   R  F  S  G   S  R  S   G  T  D   F  T  L
      agtcccttct cgcttctctg gtagccgttc cgggacggat ttcactctga
      tcagggaaga gcgaagagac catcggcaag gccctgccta aagtgagact

      T  I  S  S   L  Q  P   E  D  F   A  T  Y  Y    Sph1
      ccatcagcag tctgcagccg gaagacttcg caacttatta ctg / catgc
      ggtagtcgtc agacgtcggc cttctgaagc gttgaataat gacgtac / g

                                                Sph1      L
      ------SACB cassette(2 kb)------- -------g  / catgcctg
      ----------------------------------------cgtac  / gggac

       V  T  V   S  S  A  S   T  K  G   P  S  V   F  P  L  A
      gtcaccgtct cctcggcctc caccaagggt ccatcggtct tccccctggc
      cagtggcaga ggagccggag gtggttccca ggtagccaga aggggggaccg

        P  S  S   K  S  T   S  G  G  T   A  A  L   G  C  L
      accctcctcc aagagcacct ctgggggcac agcggccctg ggctgcctg
      tgggaggagg ttctcgtgga gacccccgtg tcgccgggac ccgacggag
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 V  K  D   Y  F  P  E   P  V  T   V  S  W   N  S  G  A
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cagttcctga tgaaggggct tggccactgc cacagcacct tgagtccgcg

  L  T  S   G  V  H   T  F  P  A   V  L  Q   S  S  G
cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag tcctcaggac
ggactggtcg ccgcacgtgt ggaagggccg acaggatgtc aggagtcctg

L  Y  S  L   S  S  V   V  T  V   P  S  S  S   L  G  T
tctactccct cagcagcgtg gtgaccgtgc cctccagcag cttgggcacc
agatgaggga gtcgtcgcac cactggcacg ggaggtcgtc gaacccgtgg

 Q  T  Y   I  C  N  V   N  H  K   P  S  N  T   K
cagacctaca tctgcaacgt gaatcacaag cccagcaacac caag
gtctggatgt agacgttgca cttagtgttc gggtcgttgtg gttc

SalI
   V  D  K   K  V  E  P   K  S  C   D  K  T   H  T  G  G
g /tcgacaaga aagttgagcc caaatcttgt gacaaaactc acacaggagg
cagct /gttct ttcaactcgg gtttagaaca ctgttttgag tgtgtcctcc


  S  G  S   A  G  G   L  N  D  I   F  E  A   Q  K  I
ttctggcagc gccggtggcc tgaacgatat ttttgaagcg cagaaaattg
aagaccgtcg cggcctccgg acttgctata aaaacttcgc gtcttttaac

                          Sal1
E  W  H  E   *  *           V  D   K  K  V   E  P  K  S
aatggcatga atgatgacga agctg /tcgac aaaaaggtgg aacctaagag
Ttaccgtact tactactgct tcgacagct/ g tttttccacc ttggattctc

                          Bsa1
  C  D  K   T  H  T   *  *           V  D  K   K  V  E
ctgcgataag acccatacct aataaggtct ca/ tcgacaaa aaggtcgaac
gacgctattc tgggtatgga ttattccaga gtagct/ gttt ttccagcttg

P  K  S  C   D  K  T   H  T  G   G  S  H  H   H  H  H
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 H  *  *                                    Bgl11
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