

pFUSE-HEAVY Vector

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto
Description	The pFUSE-HEAVY vector is a derivative of the pFUSEss-CHIg-mG1 vector (Invivogen). It is a mammalian expression vector for human/mouse hybrid IgG production and is used in a co-transfection with pFUSE-LIGHT. The expressed IgG is a hybrid human FAB from Library E or F, human hinge region, and a mouse Fc
Antibiotic resistance	Zeocin 25 ug/ml from 100mg/ml stock solution
Promoter	hEF1-HTLV Promoter
Cloning Method	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 Afe1 / Sph1 linearized vector. Insertion of target sequence involves replacement of SacB gene stuffer sequence, which provides for negative selection of the plasmid on 5% sucrose.
Initiation Codon	ATG in vector
N – terminal fusion sequence, IL2 signal peptide	MYRMQLLSICIALSLALVTNSEVQ----
C – terminal fusion sequence, identical to human FAB	--- LTVVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFIAPPKPKDVLITITLTPKVTCTVVVDISKD DPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELP MHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAP QVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWN GQPAENYKNTQPIMDTDGSYFVYSKLVQKSNWEAGN TFTCSVLHEGLHNHHTKSLSHSPGK
Termination codons	TGA in vector
Additional features	
Preferred Hosts	HEK293
5' primer for amplification of insert Fwd-HV2	5' attcggaggttcagctggtggag 3'
3' primer for amplification of insert Rev-HV2	5' gagacggtgaccagggttc 3'
5' sequencing primer pFUSE-FWD	5' acagatccaagctgtgacc 3'
3' sequencing primer ScreenSeqRevHeavy	5' agtagtccttgaccaggcag 3'

hEF1-HTLV Promoter

tctgcgccgt tacagatcca agctgtgacc ggcgcctacc tgagatcacc ggccaaggag
agacgcggca atgtctaggt tcgacactgg ccgcggatgg actctagtgg ccgcttcctc

IL2ss

M Y R M Q L L S C I A L S L A L V T
ggccaccatg tacaggatgc aactcctgtc tgcattgca ctaagtcttg cacttgtcac
ccgggtgtac atgtcctacg ttgaggacag aacgtaacgt gattcagaac gtgaacagtg

EcoR1

AfeI

SphI

N S E V Q -----SACB gene (2kb)----- **L V T V S**
gaattcggag gttcagc/gct----- gcatg/ctgg tcaccgtctc
cttaagcctc caagtcg/cga----- c/gtacggacc agtggcagag

FAB from human IgG gamma 1

S A S T K G P S V F P L A P S S K S T S
ctcggcctcc accaagggtc catcggctctt cccctggca ccctcctcca agagcacctc
gagccggagg tggttcccag gtagccagaa gggggaccgt gggaggaggt tctcgtggag

G G T A A L G C L V K D Y F P E P V T V
tgggggcaca gggccctgg gctgctgggt caaggactac ttccccgaac cggtgacggt
acccccgtgt cgccgggacc cgacggacca gttcctgatg aaggggcttg gccactgcca

S W N S G A L T S G V H T F P A V L Q S
gtcgtggaac tcaggcggcc tgaccagcgg cgtgcacacc ttcccggtg tcctacagtc
cagcaccttg agtccgctgg actggctgcc gcacgtgtgg aagggccgac aggatgacag

S G L Y S L S S V V T V P S S S L G T Q
ctcaggactc tactccctca gcagcgtgggt gaccgtgcc tccagcagct tgggcacca
gagtctgag atgagggagt cgctgcacca ctggcacggg aggtcgtcga acccgtgggt

T Y I C N V N H K P S N T K V D K K V E
gacctacatc tgcaacgtga atcacaagcc cagcaacacc aaggtcgaca agaaagtga
ctggatgtag acgttgcaact tagtgttcgg gtcgttgtgg ttccagctgt tctttcaact

Human Hinge

P K S C D K T H T C P P C P A P E L L G
gccccaaatct tgtgacaaaa ctcacacatg cctccatgt ccagcccctg aacttctggg
cgggtttaga aactgtttttagtgtgtac gggaggtaca ggtcggggac ttgaagacc

Mouse FC

G P S V F I F P P K P K D V L T I T L T
aggaccttct gtcttcatct tcccccaaa gcccaaggat gtgctacca ttactctgac
tcttgggaaga cagaagtaga aggggggttt cgggttctca cacgagtgg atagagactg

P K V T C V V V D I S K D D P E V Q F S
tcctaaggctc acgtgtgttg tggtagacat cagcaaggat gatcccagg tccagtccag
aggattccag tgcacacaac accatctgta gtcgttctca ctagggctcc aggtcaagtc

W F V D D V E V H T A Q T Q P R E E Q F

ctggtttgta gatgatgtgg aggtgcacac agctcagacg caaccccggg aggagcagtt
gaccaaacat ctactacacc tccacgtgtg tcgagctctgc gttggggccc tctctgtcaa

N S T F R S V S E L P I M H Q D W L N G

caacagcaact ttccgctcag tcagtgaact tcccatcatg caccaggact ggctcaatgg
gttgtcgtga aaggcgagtc agtcacttga agggtagtac gtggtcctga ccgagttacc

K E F K C R V N S A A F P A P I E K T I

caaggagttc aatgcaggg tcaacagtgc agctttccct gccccatcg agaaaacat
gttctcaag tttacgtccc agttgtcacg tcgaaagga cggggtagc tcttttgga

S K T K G R P K A P Q V Y T I P P P K E

ctccaaaacc aaaggcagac cgaaggtcc acaggtgtac accattccac ctcccaagga
gaggttttg tttccgtctg gcttccgagg tgtccacatg tgtaaggtg gagggttcct

Q M A K D K V S L T C M I T D F F P E D

cgagatggcc aaggataaag tcagtctgac ctgcatgata acagacttct tccctgaaga
cgtctaccgg ttcctatttc agtcagactg gacgtactat tgtctgaaga agggacttct

I T V E W Q W N G Q P A E N Y K N T Q P

cattactgtg gattggcagt ggaatgggca gccagcggag aactacaaga aactcagcc
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I M D T D G S Y F V Y S K L N V Q K S N

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W E A G N T F T C S V L H E G L H N H H

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T E K S L S H S P G K *

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SV40 polyA

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>pFUSE-HEAVY (6510bp)

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