

pFUSE-Light Vector

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto
Description	The pFUSE-LIGHT vector is a derivative of the pFUSE2ss-CLlg-mk vector (Invivogen). It is a mammalian expression vector for production of human light chain gamma 1 IgG and is used in a co-transfection with pFUSE-HEAVY. The expressed IgG is a hybrid of human FAB from Library E or F, human hinge region, and a mouse Fc region.
Antibiotic resistance	Blastocidin S 100 ug/ml from 10mg/ml stock solution
Promoter	hEF1-HTLV composite 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 / Kpn1 linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.
Initiation Codon	ATG in vector
N – terminal fusion sequence, IL2 signal peptide	MYRMQLLSICIALSLALVTNSDIQMTQSPSSLSASV GDRVTITCRASQSVSSA----
C – terminal fusion sequence, identical to human FAB	--GTKVEIKRTVAAPSVFIFPPSDSQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSLSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
Termination codons	TAG in vector
Additional features	
Preferred Hosts	HEK293
5' primer for amplification of insert Light-Fwd-Afe1	5' cagtcggtgccagcgcctg 3'
3' primer for amplification of insert Rev-LV2	5' ttgatctccaccttggtac 3'
5' sequencing primer pFUSE-FWD	5' acagatccaagctgtgacc 3'
3' sequencing primer SeqscreenRevligh	5' acctccactgtactttgg 3'

hEF1-HTLV Promoter

acgtctttgt ttcgttttct gttacagatc caagctgtga ccggcgccta cctgagatca
tgcagaaaca aagcaaaaga caatgtctag gttcgcact ggccgcgat ggactctagt

IL2ss EcoRI
M Y R M Q L L S C I A L S L A L V T N
acatgtacag gatgcaactc ctgtcttgca ttgcactaag tcttgactt gtcacgaatt
tgtacatgtc ctacgttgag gacagaacgt aacgtgattc agaactgaa cagtgttaa

S D I Q M T Q S P S S L S A S V G D R V
cagatatcca gatgaccag tccccgagct cctgtccgc ctctgtggg gatagggta
gtctataggt ctactgggtc aggggctcga gggacaggc gagacaccg ctatccagt

AfeI
T I T C R A S Q S V S S A V A W Y Q Q K
ccatcacctg ccgtgccagt cagtccgtgt ccagc/gctgt agcctggat caacagaaac
ggtagtggac ggcacggta gtcaggcaca ggtcg/cgaca tcggaccata gttgtctttg

HindIII KpnI
P G K A P K L G T K V E I K
caggaaaagc tccgaagctt-----SACB gene(2kb)----- ggtac/caagg tggagatcaa
gtccttttcg aggcctcgaa-----c/catggttcc acctctagt

R T V A A P S V F I F P P S D S Q L K S
acgaactgtg gctgcaccat ctgtcttcat cttcccccca tctgattcac agttgaaatc
tgcttgacac cgacgtggta gacagaagta gaagggcgga agactaagtg tcaactttag

G T A S V V C L L N N F Y P R E A K V Q
tggaactgcc tctgtttgtg gctgtctgaa taacttctat cccagagagg ccaaagtaca
accttgacgg agacaacaca cggacgactt attgaagata gggctctctcc ggtttcatgt

W K V D N A L Q S G N S Q E S V T E Q D
gtggaagggtg gataacgcc tccaatcggg taactcccag gagagtgtca cagagcagga
caccttccac ctattgcggg aggttagccc attgagggtc ctctcacagt gtctcgtcct

S K D S T Y S L S S T L T L S K A D Y E
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K H K V Y A C E V T H Q G L S S P V T K
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NheI sv40 polyA
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>pFUSE-LIGHT (5983bp)

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