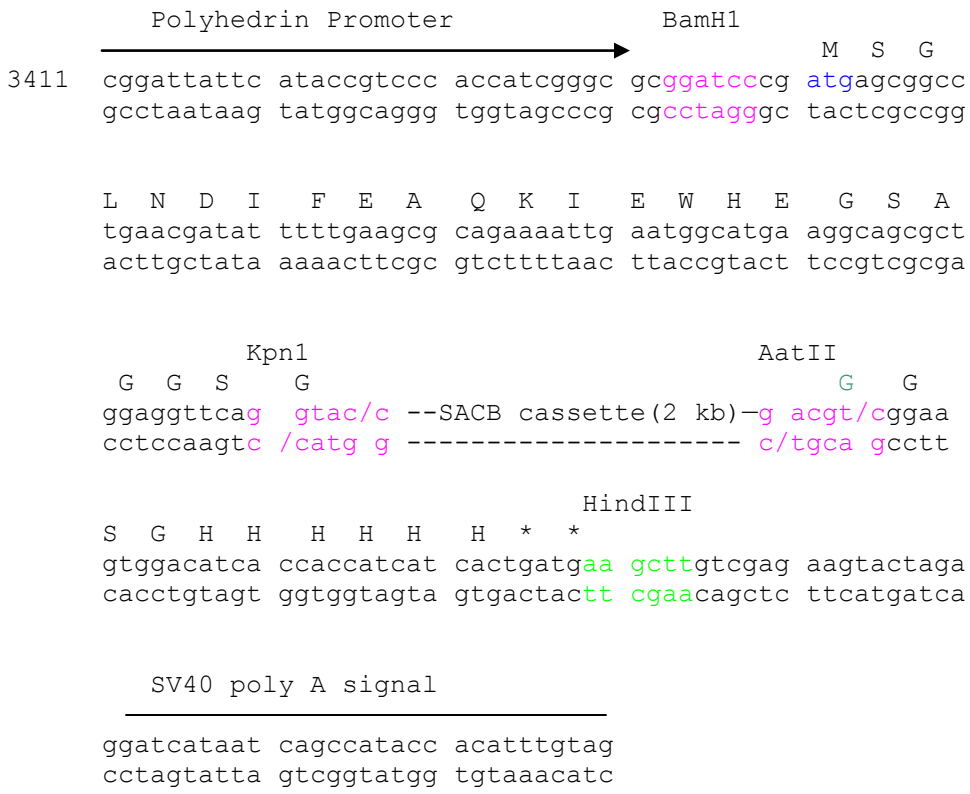
pFBD-BirA Vector

Source	Constructed by Peter Loppnau
Company	Structural Genomics Consortium, Toronto

Description	The pFBD-BirA expression vector is a derivative of pFastBac Dual vector (Invitrogen). It is used for the production of in vivo biotinylated proteins in insect cells. The Polyhedrin promoter drives expression of the target protein with the addition of an N-terminal fusion containing the AviTag biotinylation signal and a C-terminal Hexa His-tag. The p10 promoter drives expression of the birA gene.

Antibiotic resistance	Ampicillin 100 ug/ml, Gentamycin
Promoters	Polyhedrin and p10
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 Kpn1 and AatII 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	After BamH1 site in vector
N – terminal fusion	MSGLNDIFEAQKIEWHEGSAGGSG--
C – terminal fusion	--GGSGHHHHHH
Termination codons	TGATGA stop codons are included in the vector after His tag.
Additional features	The E. coli birA gene under control of the p10 promoter encodes for a 35.3-kDa 321 amino acid biotin ligase protein that transfers biotin to the AviTag.
Prefered Hosts	BEVS
5’ primer for amplification of insert	5’ agcgctggaggttcaggt --- 3’
3’ primer for amplification of insert	5’ tgatgtccacttccgcc --- 3’
5’ sequencing/ screening primer pFBOH-FWD	5’ CCGGATTATTCATACCGTCCCACCA 3’
3’ sequencing/ screening primer pFBOH-REV	5’ CTGATTATGATCCTCTAGTACTTCT 3’

pFBD-BirA cloning / expression region



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Notes.

Can screen colonies by miniprep and digestion with BamH1/HindIII

Infusion enzyme has 3’ to 5’ exonuclease activity so the Kpn1 and AatII enzymes were chosen for their 3’ overhangs, that are removed and allow seamless cloning.