

Resource Summary Report

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pFastBac1-melHis10TEV

RRID:Addgene_159428

Type: Plasmid

Proper Citation

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Plasmid Information

URL: <http://www.addgene.org/159428>

Proper Citation: RRID:Addgene_159428

Bacterial Resistance: Ampicillin

Vector Backbone Description: Backbone Marker:Invitrogen; Backbone Size:4682;
Vector Backbone:pFastBac1; Vector Types:Insect Expression; Bacterial
Resistance:Ampicillin

Comments: - This vector is designed to avoid adding non-native amino acid residues to the protein of interest, following TEV protease cleavage of the N-terminal purification tags. Subcloning requires ligation-independent cloning and use of the two BseRI restriction sites that are present in this empty vector. The two BseRI sites span a removable 432 nucleotide sequence. Cloning into the BseRI-digested vector can be achieved using a ligation-independent system that is dependent on homologous recombination, such as the Takara In-Fusion HD Cloning Plus system, which requires simply designing the PCR-derived insert (containing the protein of interest) to contain 15 bp at each end that matches the 15 bp at each end of the linearized parent vector. In other words, add these 15 bp, AACCTCTACTTCCAA, to the 5' end of the PCR FORWARD primer, and add these 15 bp, AGTACTTCTCGACAA, to the 5' end of the PCR REVERSE primer. - The sequence of the N-terminus will be MKFLVNVALVFMVVYISYIYA + AAPE + HHHHHHHHHH + GAP + ENLYFQ*X, where * is the TEV protease cleavage site and X is the N-terminus of the protein of interest. Note that TEV protease cleaves most efficiently when X = S, G, A, M; for other compatible residues see Kapust et al 2002, PMID 12074568. - The first 21 residues of the N-terminus (MKFLVNVALVFMVVYISYIYA) is the melittin signal peptide, which directs the protein to the endoplasmic reticulum for potential secretion. These 21 residues are expected to be removed by the cell's signal peptidase. - BseRI cuts 8-10 nucleotides outside of its own recognition sequence and is a non-palindromic site. The placement & orientation of the two BseRI sites eliminates all of the BseRI recognition sequence in the resulting subclone. - An unwanted BseRI site was removed from the parent pFastBac1 vector by introducing a silent mutation (CTC to CTG)

in a leucine codon in the gentamicin-resistance gene. - To identify colonies containing a cloned insert, used the primers PFASTBAC1F (CTAGTGGTTGGCTACGTATACTCCG) and PFASTBAC1R (GGACAAACCACAACCTAGAATGCAGTG). - To verify the PCR-generated insert sequence, use sequencing primers POLYHEDF (AAATGATAACCATCTCGC), SV40PAR (GAAATTTGTGATGCTATTGC). - To verify Tn7-based incorporation of the expression construct into the bacmid DNA in the E. coli strain DH10Bac, use PCR primers BACM13F (CCCAGTCACGACGTTGTAAAACG) and BACM13R (AGCGGATAACAATTTTCACACAGG). A bacmid containing the correct insert has a PCR product size of ~2300 base pairs plus the length of the added insert.

Plasmid Name: pFastBac1-melHis10TEV

Record Creation Time: 20220422T221903+0000

Record Last Update: 20260815T080827+0000

Ratings and Alerts

No rating or validation information has been found for pFastBac1-melHis10TEV.

No alerts have been found for pFastBac1-melHis10TEV.

Data and Source Information

Source: [Addgene](#)

Usage and Citation Metrics

We have not found any literature mentions for this resource.