Resource Summary Report

Generated by RRID on Aug 26, 2026

pelBHisTEV

RRID:Addgene_157741
Type: Plasmid

Proper Citation

RRID:Addgene_157741

Plasmid Information

URL: http://www.addgene.org/157741

Proper Citation: RRID:Addgene_157741

Bacterial Resistance: Kanamycin

Vector Backbone Description: Backbone Size:5401; Vector Backbone:pelB-MBP (DNASU access. no. EvNO00813783); Vector Types:Bacterial Expression; Bacterial Resistance:Kanamycin

Comments: - Empty vector (kanamycin-R) adds TEV-protease-removable N-terminal PelB signal sequence + His10, plus potential C-term His6. Non-leaky expression due to T7lac promoter. - Has an advantage in leaving no added amino acid residues following TEV protease cleavage of PelB signal sequence + His10. This advantage is made possible by cloning into the two BseRI restriction sites. - 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, AACCTGTACTTCCAA, to the 5' end of the PCR FORWARD primer, and add these 15 bp, GTGGTGGTGCTCGAG, to the 5' end of the PCR REVERSE primer. - The sequence of the added N-terminus is the PelB signal sequence (M1-A22 of GenBank #AAA24848) + 13 aa linker + His10-tag + 4 aa linker + TEV protease cleavage site. The N-terminal protein sequence is: MKYLLPTAAAGLLLLAAQPAMA + MDIGINSDPNSSS + HHHHHHHHHH + PMGS + ENLYFQ*X, where * is the TEV protease cleavage site and X is the N-terminal residue of the introduced 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. - A C-terminal His6-tag is possible: if no stop codon is included in the cloned insert, the sequence LEHHHHHH is added to the C-terminus. - The PelB signal sequence directs the

protein of interest to the E. coli inner membrane. Whether the protein traverses the inner membrane is dependent on the protein of interest. - 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. - To verify the PCR-generated insert sequence, use sequencing primers T7 (TAATACGACTCACTATAGGG), T7-Ter (GCTAGTTATTGCTCAGCGG).

Plasmid Name: pelBHisTEV

Record Creation Time: 20220422T221854+0000

Record Last Update: 20260815T080808+0000

Ratings and Alerts

No rating or validation information has been found for pelBHisTEV.

No alerts have been found for pelBHisTEV.

Data and Source Information

Source: Addgene

Usage and Citation Metrics

We have not found any literature mentions for this resource.