Vector pPconst1326 for Constitutive Protein Expression

Product Information Sheet
 # BMEG45

SUMMARY

shipped at RT; store at 4 °C

For research use only

Product

pPconst1326 is an *E. coli*/ *Bacillus* shuttle vector for the constitutive expression of recombinant proteins in *Bacillus megaterium.* pPconst1326 can be used for cloning of a target gene in *E. coli* and protein production in *Bacillus* sp..

Description

To generate the pPconst1326 constitutive vector the promoter of pyruvate dehydrogenase operon (pdhABCD, *bmd_1326-1329)* was isolated from *B. megaterium* DSM319 and cloned into an *E. coli*/ *Bacillus* shuttle vector. The promoter region is followed by the native ribosomal binding site (RBS) of the corresponding gene cluster including its start codon that is located upstream of multiple cloning site (MCS). First restriction site (SpeI) allows cloning of a gene of interest fused to nine additional nucleotides encoding Met-Thr-Ser at its N-terminus. The pPconst1326 carries the *oriU* and *repU* gene from *Bacillus cereus* for replication in *Bacillus sp.*, the *tetL* gene providing resistance against tetracycline (tetracycline efflux pump), and functional genetic elements for *E. coli* (origin of replication ColE1 *ori* and the *bla* gene encoding a β-lactamase which provides resistance against ampicillin). Unlike inducible gene expression system the expression of the target protein is driven constitutively by the pdhABCD promoter. Thus, addition of an inducer is not required during the cultivation of *B. megaterium* cells.

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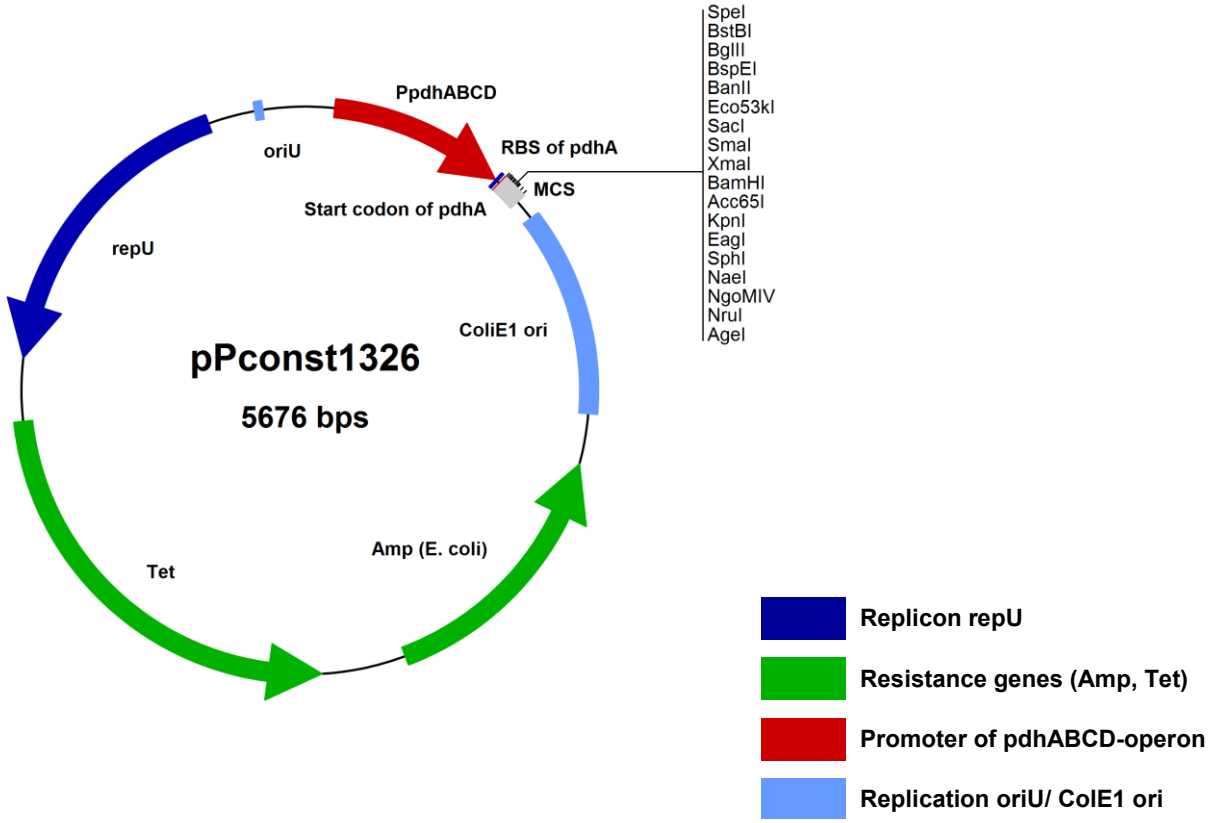
Vector Map



Handling Instructions

For propagation of the pPconst1326 plasmid, transform *E. coli* DH10B cells with 10-50 ng of vector DNA (reconstituted in distilled water or 10 mM Tris/HCl buffer, pH 8.5). Detailed protocols for *E. coli* molecular genetic handling (growth, transformation, plasmid preparation, etc.) can be found in the relevant laboratory manuals such as Green and Sambrook (2012). After transformation, single colonies carrying pPconst1326 plasmid can be selected by plating the cells on LB agar plates containing 100 µg/ml of ampicillin.
After preparing the plasmid DNA from the *E. coli*, proceed restriction digestions and ligation of your gene of interest into pPconst1326 using appropriate cloning sites. Transform *E. coli* cells with the ligation product and plate subsequently onto LB agar plates containing ampicillin. Verify several clones by restriction digestions and sequencing analysis. After confirming of a successful cloning *B. megaterium* can be transformed with the plasmid containing your target gene and grown on selecting medium with 10 µg/ml of tetracycline at 30 °C overnight. Streak out several single colonies onto fresh agar plates and use them for protein production. Required protocols for *B. megaterium* transformation and protein expression have been precisely described in MoBiTec's handbook on "*Bacillus megaterium* Protein Production System". It is available for download on our website at www.mobitec.com.

Order Information, Shipping and Storage

Order#	Product	Amount
BMEG45	*Bacillus megaterium* vector pPconst1326, constitutive	10 µg
Lyophilized DNA, shipped at RT, store at 4 °C Once the DNA has been dissolved in sterile water or TE buffer we recommend long-term storage at -20 °C.		