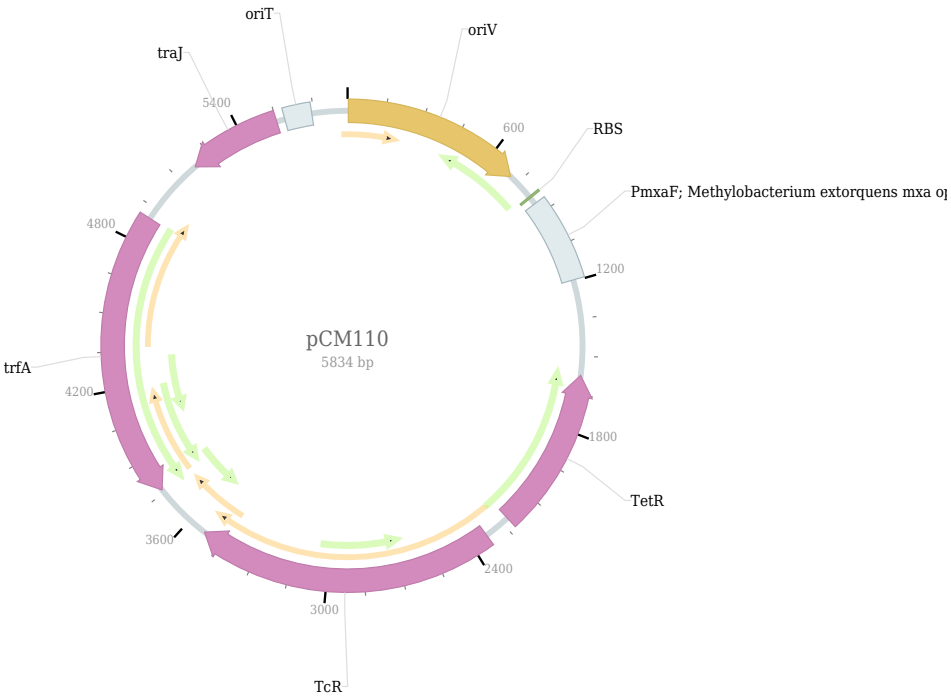
pCM110

Vector ID	V008445
Antibiotic Resistance	Tetracycline
Length	5834 bp
Vector Type	Cloning vector

Product Information

Replication Origin	oriV
Source/Author	Marx CJ, Lidstrom ME.
Growth Strain(s)	DH5a
Comment	The plasmid pCM110 is an expression vector specifically developed for use in Methylobacterium extorquens AM1. It is constructed based on an IncP plasmid that lacks both the ColE1 ori and Plac. The construction of pCM110 involves inserting the 0.4 kb HindIII - NsiI fragment of pCM27 containing Pmax into pCM60 (C. J. Marx & M. E. Lidstrom, unpublished results) which had been cut with HindIII and NsiI. This expression vector provides minimal expression in E. coli, which is advantageous for the introduction of toxic genes into M. extorquens AM1. For example, a construct containing xylE cloned into pCM110 (pCM111) was created to compare its XylE expression level to that of another vector (pCM81) in both organisms. The results showed that pCM111 provided a high level of expression in M. extorquens AM1, 1.5 - 2-fold higher than that achieved with pCM81; however, extracts from E. coli JM109 bearing pCM111 had a basal level of XylE activity, nearly at the background. These results indicate that the Pmax is expressed at very low levels in E. coli. Therefore, while other expression vectors like pCM80 are useful for the expression of most cloned genes, pCM110 may be required to express genes that are toxic in E. coli.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.