Vector pMMEc4

Product Information Sheet
PEC04

SUMMARY

shipped at RT; store at 4 °C

For research use only

Product

pMMEc4 is a helper plasmid for enabling the repression of the P_{xylA} promoter of a second *E. coli*/ *Bacillus* shuttle vector. pMMEc4 replicates in *E. coli* and carries the *xylR* gene under control of arabinose P_{BAD} promoter.

Description

The construction of expression vectors for Gram-positive bacteria is often done by using shuttle vectors (e.g., *E. coli*/ *B. megaterium*). Since transformation of *E. coli* is easy, fast, and successful it is a common host for molecular cloning. In case of genes encoding toxic products, it is essential to prevent any expression within *E. coli* during cloning procedures.
The xylose inducible *xylA* / *xylR* induction system is not very tightly regulated in *E. coli.* It shows some degree of expression even without addition of an inducer. The vector pMMEc4 was constructed as a helper plasmid for preventing any expression from the *xylA* promoter in *E. coli*. It encodes for the repressor protein XylR that prevents the gene expression driven by the P_{xylA} promoter. The expression of *xylR* is controlled by arabinose P_{BAD} promoter and the AraC protein. In the presence of 0.2% arabinose, the AraC protein will bind to operator sequence that activates expression of the *xylR* gene, and additionally upregulate its own expression. The vector pMMEc4 carries the p15A origin of replication that is compatible with vectors from other incompatibility groups, like ColE1 group.

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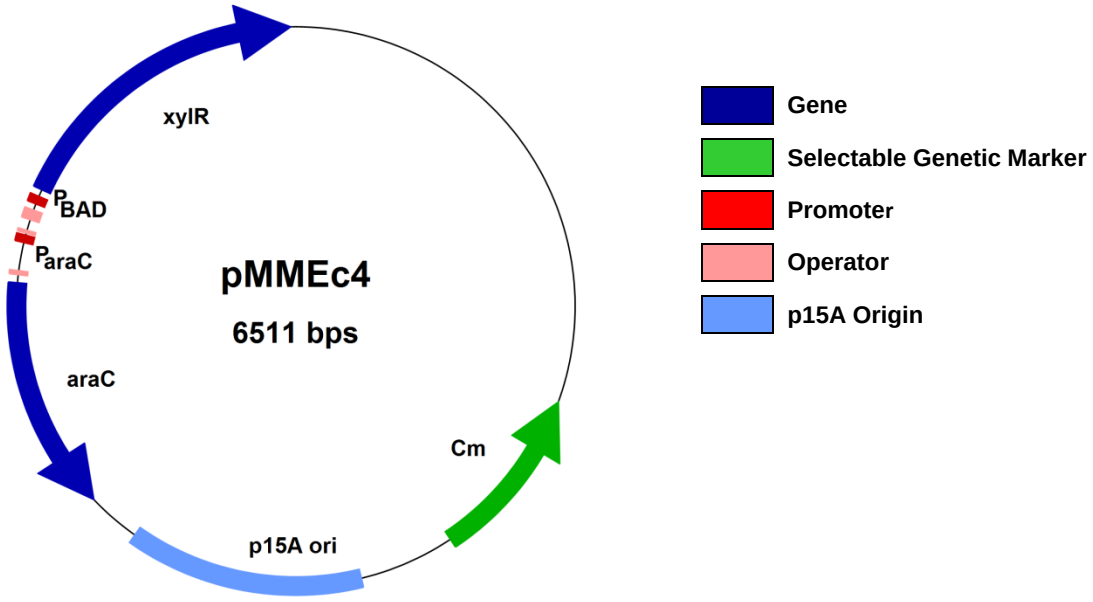
Vector Map



Handling Instructions

For propagation and use of pMMEc4, transform *E. coli* cells with 1-50 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, etc.) can be found in the relevant laboratory manuals such as Sambrook and Russell (2001). After transformation, bacterial colonies with pMMEc4 vector can be selected by plating the cells on LB plates containing 30 µg/ml of chloramphenicol.

Ligate your gene of interest into the *E. coli*/ *B. megaterium* shuttle vector under control of the xylose inducible xylA/xylR induction system and transform the ligation product into *E. coli* cells. Selection plates should contain 30 µg/ml of chloramphenicol and a second antibiotic corresponding to the resistance gene of the shuttle vector and 0.2% arabinose. The latter is essential for a successful repression of the xylA promoter of the shuttle vector.

For transforming a *Bacillus* strain e.g., *B. megaterium* with the shuttle vector construct propagated in *E. coli*, the whole plasmid DNA (including pMMEC4) has to be prepared and used for transformation. Since pMMEC4 cannot replicate in Gram-positive bacteria, it will get lost. Screening for clones carrying the shuttle expression vector with the gene of interest can be done by using the appropriate antibiotic.

Quality Warranty

DNA concentration and purity was checked by UV spectrophotometry. The functional sequences were checked by sequence analysis.

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Order Information, Shipping, and Storage

Order#	Product	Amount
PEC04	pMMEC4, lyophilized DNA	10 µg
shipped at room temperature (RT); store at 4 °C. Once the DNA has been dissolved in sterile water or TE buffer we recommend storage at -20 °C.		

Related Products

Order#	Product	Amount
BMEG10	*Bacillus megaterium vector*, pMM1522, lyophilized DNA	10 µg
BMEG11	*Bacillus megaterium* vector, pMM1525, lyophilized DNA	10 µg
BMEG12	*Bacillus megaterium* vector, pHIS1522, lyophilized DNA	10 µg
BMEG13	*Bacillus megaterium* vector, pHIS1525, lyophilized DNA	10 µg
BMEG15	*Bacillus megaterium* vector pSTREPHIS1525	10 µg
BMEG30	*Bacillus megaterium* vector p3STOP1623hp	10 µg
BMEG33	*Bacillus megaterium* vector pSP_{LipA}-hp	10 µg
BMEG34	*Bacillus megaterium* vector pSP_{YocH}-hp	10 µg
BMEG36	*Bacillus megaterium* vector pC-STREP1623hp	10 µg
BMEG02	*Bacillus megaterium* protoplast, strain WH320	5 x 500 µl
BMEG50	*Bacillus megaterium* protoplasts, strain MS941	5 x 500 µl
CB-CEC30100-01	QuickCells Competent *E. coli*	20 x 50 µl
A1414-25GMAG	Ampicillin, sodium salt	25 g
ENZ-286-1PS	Recombinant T4 DNA Ligase	20,000 U
GE-TLK0110-1	TurboLigation™ Kit	100 rxn

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