pBBR RESO vector, lyophilized DNA

Product Information Sheet
 # RESO01

SUMMARY

shipped at room temperature; store at 4 °C

For research use only

Product Description and Application

- the promoter sequence of a cloned gene or operon
- promoter sequences in random tnpR-gene fusion libraries
- genes induced under harsh environmental conditions, such as various stresses, where other reporter genes and selection systems (cat, bla) cannot be used

The plasmid pBBR RESO is a broad-host-range promoter cloning vector. In contrast to other known broad-host-range vectors, it is maintained at a medium copy number and has a reasonable size of about 6.8 kb. It stably replicates in any gram-negative bacterium studied, and is therefore particularly interesting for the isolation and genetic analysis of DNA sequences with promoter activity in the homologous organism. The reporter system used employs the resolvase-mediated excision of a kanamycin (Kan)-resistance gene flanked by two res sequences. Cloning an active promoter results in Kan-sensitive clones.

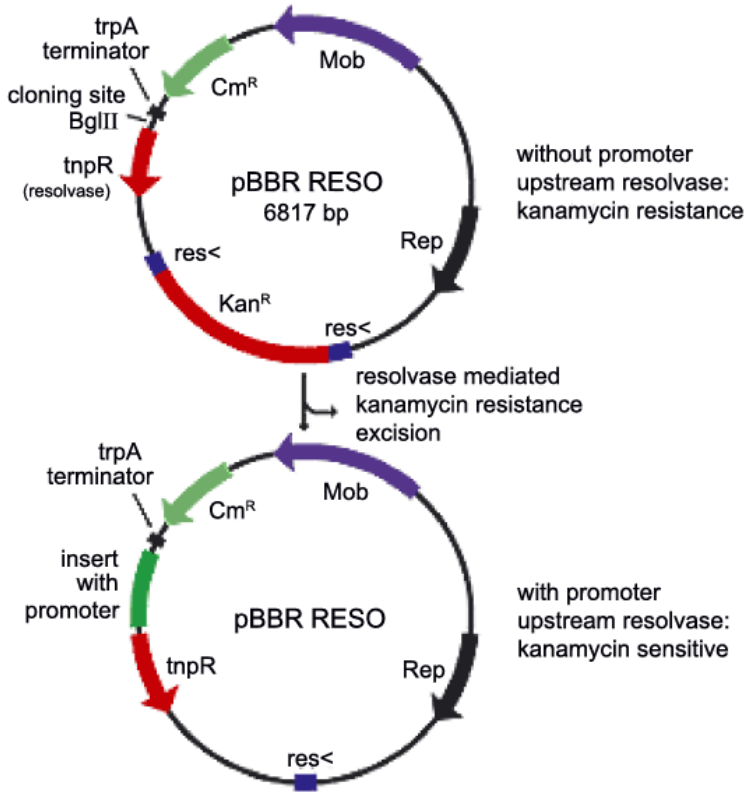
pBBR RESO was derived from pBBR1MCS[3], which itself is a modification of the broad-host-range plasmid pBBR1CM (Antoine & Locht, 1992). It contains a chloramphenicol resistance gene (CM) and a unique *BglII* cloning site immediately upstream the promoterless reporter gene tnpR, encoding the resolvase from transposon Tn3[4]. Two directly repeated res sequences flanking the Kan-gene are located downstream of tnpR[2]. A transcriptional fusion between a DNA fragment cloned into BglII and tnpR results in expression of the latter, and resolvase-mediated strand exchange occurs between the res sites. This leads to the irreversible shift from a Kan-resistant to a Kan-sensitive phenotype of the host bacterium. Clones should be plated on Cm-containing agar and assayed for kanamycin resistance/sensitivity. The only requirement for the use of this system is a resolvase-free background, i. e. the gram-negative strains should not contain any transposon potentially coding for resolvase. Besides BglII the DNA of interest can also be digested with Sau3A or BamHI, since the overhangs are compatible.

A major advantage of the system lies in the fact that not only constitutively but also transiently induced promoters can be detected. Further, the screening for promoter-containing clones does not necessitate a selection pressure onto the reporter gene product.

Host Strains

- **Plasmid propagation:** transposase-free *E. coli* strains, such as DS941 (not available at MoBiTec)
- **Expression:** a transposase-free gram-negative host (see pBBR122 in MoBiTec catalog chapter 4.2. for strains tried so far with pBBR derivatives)

Vector Map



Promoter Cloning Vector pBBR RESO

Cloning a promoter sequence into the BglII site causes resolvase mediated kanamycin (Kan) excision and, thus, irreversible Kan sensitivity. Mob is involved in mobilization, Rep in replication. TnpR: resolvase. Cm^R: chloramphenicol resistance.

References

Antonie, R. and C. Locht, *Mol. Microbiol*. 6 (1992) 1785-179
Blake, D.G. *et al., Current Biology* 5 (1995) 1036-1046
Kovach, M.E. *et al., BioTechniques* 16 (1994) 800-802
Stark, W.M. *et al., Cell* 58 (1989) 779-790

The vector pBBR RESO has been developed by S. Köhler, J. Teyssier and J.P. Liautard at the INSERM laboratory U-431, Montpellier.

Order Information, Shipping and Storage

Order#	Product	Quantity
RESO01	pBBR RESO vector, lyophilized DNA	5 µg
shipped at room temperature; store at -20 °C		