

Improved Mycobacterial Tetracycline Inducible Vectors

A range of improved tetracycline inducible vectors to study the function of essential mycobacterial genes.

Proposed use

Conditional gene expression systems are powerful tools for studying essential genes in bacteria. Tetracycline and its analogues are ideal inducer molecules as they regulate gene expression at very low concentrations (nM range), show good bio-availability (ie. they can penetrate both mycobacterial and animal cells), are non-toxic at the necessary levels and are stable over the required length of study.

Problem addressed

There is a lack of genetic tools available to researchers to study the function of essential mycobacterial genes, we have therefore constructed a range of improved tetracycline inducible vectors. We have shown that changing the terminators upstream of the inducible promoter does not affect background transcription, and that repressor binding to the operator is a more critical step in controlling regulation. We have eliminated further read-through problems by changing the backbone vector used. The TetRO promoter can be induced in a dose-dependent manner by tetracycline, anhydrotetracycline and doxycycline.

Technology overview

pMEND-Lx	pMIND-Lx with Gly4o to Thr4o change in TetR protein; episomal
pMEND-Lx-Int	Integration version of pMEND-Lx
pKWo8-Lx	TetRO promoter from pMEND-Lx inserted into the backbone of pSE100; episomal
pKWo8-Lx-Int	Integration version of pKWo8-Lx

Plasmids available

- pMEND-Lx
- pMEND-Lx-Int
- pKWo8-Lx
- pKWo8-Lx-Int

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Additional information

Can be purchased for academic use through Addgene: <u>https://www.addgene.org/browse/article/3409/</u>.

Link to published paper(s)

Williams KJ, Joyce G, Robertson BD. Improved mycobacterial tetracycline inducible vectors. Plasmid. 2010 Apr 29.

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