

Strain	Location of antibiotic resistance cassette insertion	Background	Antibiotic Resistance
Tn::Rv1625c	Mycomar transposon insertion at 904bp of <i>Rv1625c</i>	<i>M. tuberculosis</i> CDC1551	Kanamycin
ΔRv1625c	(92-1256bp) of <i>Rv1625c</i>	<i>M. tuberculosis</i> CDC1551	Hygromycin
ΔMt-Pat	(21-981bp) of <i>Rv0998</i>	<i>M. tuberculosis</i> CDC1551	Hygromycin

  

Strain	Plasmid Constructs	Background	Antibiotic Resistance
2xRv1625*	pMV306+aacC4 (apramycin resistance)+ hsp60'::Rv1625c (1-1329bp)	WT <i>M. tuberculosis</i> CDC1551	Apramycin
Comp <sub>Full</sub> *	pMV306 + aacC4 (apramycin resistance) + hsp60'::Rv1625c (1-1329bp)	ΔRv1625c	Hygromycin, Apramycin
Comp <sub>D204</sub> *	pMV306+aacC4 (apramycin resistance) + hsp60'::Rv1625c (1-612bp)	ΔRv1625c	Hygromycin, Apramycin
TetOn-cAMP**	pDE1264 (pDO23A+Rv1264 (627-1191bp) ) + pEN41A-T10M + pEN12A-p606 + pDE43-MEH	WT <i>M. tuberculosis</i> CDC1551	Hygromycin
TetOn-Rv1264 <sub>D265A</sub> **	pDE1264 <sub>D265A</sub> (pDO23A+Rv1264 <sub>D265A</sub> (624-1191bp) )+ pEN41A-T10M + pEN12A-p606 + pDE43-MEH	WT <i>M. tuberculosis</i> CDC1551	Hygromycin
prpD'::GFP	pCherry3 + kanamycin resistance + prpD'::GFP + smyc'::mCherry	WT <i>M. tuberculosis</i> CDC1551	Kanamycin
ΔRv1625c prpD'::GFP	pCherry3 + kanamycin resistance + prpD'::GFP + smyc'::mCherry	ΔRv1625c	Kanamycin
Comp <sub>Full</sub> prpD'::GFP	pCherry3 + zeocin resistance + prpD'::GFP + smyc'::mCherry	Comp <sub>Full</sub>	Hygromycin, Apramycin, Zeocin
Comp <sub>D204</sub> prpD'::GFP	pCherry3 + zeocin resistance + prpD'::GFP + smyc'::mCherry	Comp <sub>D265A</sub>	Hygromycin, Apramycin, Zeocin
TetOn-cAMP prpD'::GFP	TetOn-cAMP + prpD'::GFP + smyc'::mCherry	WT <i>M. tuberculosis</i> CDC1551	Hygromycin
ΔMt-Pat TetOn-cAMP prpD'::GFP	TetOn-cAMP + kanamycin resistance + prpD'::GFP + smyc'::mCherry	ΔMt-Pat	Hygromycin, Kanamycin
TetOn-1264 <sub>D265A</sub> prpD'::GFP	TetOn-Rv1264 <sub>D265A</sub> + prpD'::GFP + smyc'::mCherry	WT <i>M. tuberculosis</i> CDC1551	Hygromycin

\*Apramycin gene sourced from Consaul SA, Pavelka MS Jr. Use of a novel allele of the *Escherichia coli* aacC4 aminoglycoside resistance gene as a genetic marker in mycobacteria. *FEMS Microbiol Lett.* 2004 May 15;234(2):297-301. doi: 10.1016/j.femsle.2004.03.041. PMID: 15135536.

\*\*Plasmids for Gateway cloning to construct TetOn-cAMP and TetOn-Rv1264<sub>D265A</sub> developed from Klotzsche M, Ehrh S, Schnappinger D. Improved tetracycline repressors for gene silencing in mycobacteria. *Nucleic Acids Res.* 2009 Apr;37(6):1778-88. doi: 10.1093/nar/gkp015. PMID: 19174563.

**Table S3. Mtb strains used in these experiments.**