

Title:

The gene *fmt*, encoding tRNA^{fm^t}-formyl transferase, is essential for normal growth of *M. bovis*, but not for viability.

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Supplementary Material:

Supplementary data:

Supplementary table 1: oligo-primers used in this work.

Primer name	Seq	Template	product	remarks
fmtsmgup	5 – CAGCGACGAGTTCGTCGCCGAGCTCACCGA-3	<i>M. smeg fmt</i> (Msmeg_3064)	0.6kb	No product on <i>Mtbfmt</i>
fmtsmgdo	5 – CCAGCCGCACCGGATGCGACCCGGTGCCGA-3			
fmtdelchq1	5 – GTTCTTCGCGTTCTCCGTGGGATGC-3	gDNA <i>M. smeg wt</i> or Δ <i>fmt</i>	Wt: 3.2kb Δ <i>fmt</i> : 2.3kb	
fmtdelchq2	5 – GGTTGGCGCCGCCACCGTGAATCTGCGG-3			
fmtTBup	5 – GACGTGATCGCCGTGTTGACCCGTCCGGAT-3	Mtb <i>fmt</i> (Rv1406)	0.75kb	No product on <i>MSmfmt</i>
fmtTBdo	5 – TTCTTGCCGGGCGGCTGAATCTGGCCCAGC-3			
fmtKOchqD	5 – CGCCGCGCCCAACGCGTCAGCAA - 3	gDNA BCG wt or Δ <i>fmt</i>	Wt:2.0kb Δ <i>fmt</i> (hyg): 2.8kb	
fmtKOchqU	5 – TCCTGGTATTGCAGGGTCAGCC - 3			
Int end	5 – TGCGACGACCAAGGAGCTGAT - 3	Bind to the integrase and oriE of attb-integrating plasmids, including pDB284 and pDB299	Used to confirm exchange of pDB284 (1532bp product)and pDB299 (830bp)	Bind to both plasmids, but produce a product of different sizes.
oriE strat	5 – CTGACGCTCAGTGGAAGTAG - 3			
strt	5 – GTGCGCCTTGCTTTGCCGG – 3	<i>fmt</i>	0.94kb	Internal primers of <i>Mtbfmt</i>
R1	5 – GTGATGGTGTGTTGCCCGTGCGGCCAG – 3	<i>fmt</i>		
mid1	5 – CCGAACTCGGCAGAGTTCGT – 3	<i>fmt</i>	0.48kb	
mid2	5 – GGATCCGCCGCTCCACGA – 3	<i>fmt</i>		

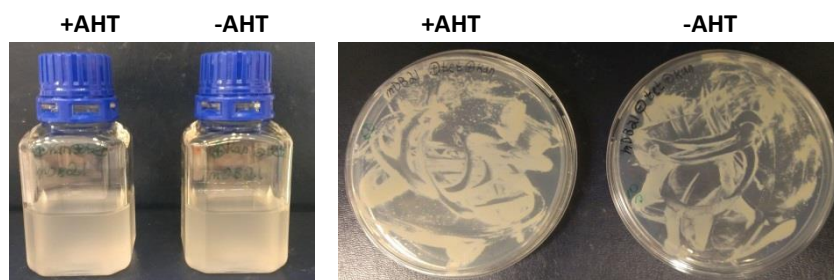
Supplementary table 2: plasmids used in this work

Plasmid name	replication	Genes/constructs	Resistance	use
pDB117	<i>Attb</i> integrating	AHT-Mtbfmt	Kanamycin	Conditional FMT expression
pDB178	No mycobacterial replication	<i>mCherry</i> , <i>galk</i> , <i>sacB</i>	Zeocin streptomycin	Two-step deletion of <i>fmt</i> in <i>M. smegmatis</i>
pYUB412	<i>Attb</i> integrating	None	Hygromycin	Removal of pDB117 from genome
pDB245	oriE (no oriM)	Allelic exchange construct for <i>fmt</i>	Hygromycin	Creation of phDB30
pDB284	<i>Attb</i> integrating	Mtbfmt	Kanamycin	Creation of Mero-diploid strain of BCG
pDB290	<i>Attb</i> integrating	Mtbfmt	Zeocin	Exchange of pDB284
pDB299	<i>Attb</i> integrating	<i>lacZ</i>	Zeocin	Complete removal of pDB284 to create full KO
pDB332	Episomal, oriM	<i>fmt</i> , <i>mCherry</i>	kanamycin	<i>fmt</i> complementation in full deletion-mutant

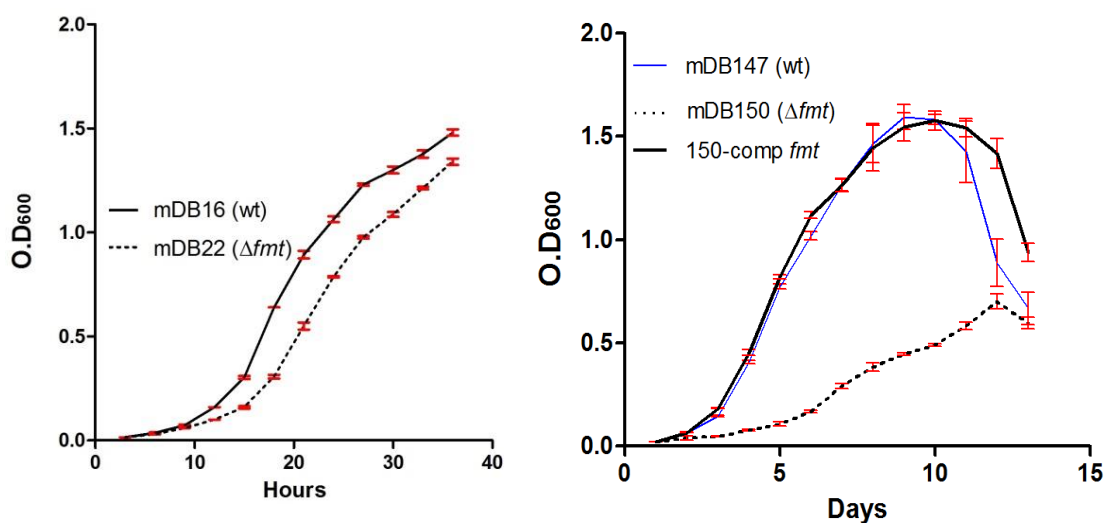
Supplementary table 3: Mutant and strain used in this work:

Name of mutant	genotype	Creation
<i>M. smegmatis</i> MC ² -155	Wild type	Lab strain
<i>M. smegmatis</i> mDB21	Δfmt , attb:AHT-Mtbfmt:kana	Insertion of pDB117 into wt, deletion of <i>fmt</i> using pDB178 two-step
<i>M. smegmatis</i> mDB22	Δfmt , attb:hyg	Exchange of pDB117 by pYUB412 in mDB21
BCG subsp. <i>russia</i>	Wild type	Lab strain
BCG mDB113	attb:fmt:kana	BCG Russia + pDB284
BCG mDB123	Δfmt :hyg, attb:fmt:kana	Deletion of <i>fmt</i> by phDB30, in mDB113
BCG mDB147	Δfmt :hyg, attb:fmt:zeo	mDB123 + pDB290
BCG mDB150	Δfmt :hyg, attb:lacZ:zeo	mDB123 + pDB299
BCG mDB168 (mDB150 comp- <i>fmt</i>)	Δfmt :hyg, attb:lacZ:zeo, plasmid OriM <i>fmt</i> :kana	mDB150 + pDB332

Supplementary figures:



Supplementary figure 1: mDB21 can grow with or without the addition of Anhydrotetracycline (AHT).



Supplementary figure 2: The linear/linear growth curve of mDB22 vs. mDB16 (right) and mDB150 vs. mDB147 vs. mDB150 comp-*fmt* (left). Error bars are Standard Error of the Mean, SEM.