

Table S1. Bacterial strains and plasmids used in this study

Strains	Description	Marker	Reference
<i>E. coli</i> TOP10	F ⁻ <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) ϕ 80/ <i>lacZ</i> Δ M15 Δ <i>lacX74 deoR recA1 araD139 Δ(<i>ara-leu</i>)7697 <i>galU galK rpsL endA1 nupG</i>; used for general cloning</i>	-	Invitrogen
<i>M. smegmatis</i>	strain mc ² 155	-	(1)
Δ MSMEG_1351	mc ² 155 MSMEG_1351:: <i>hyg</i>	Hyg	This study
<i>M. bovis</i> BCG	strain Pasteur 1173P2 (referred to as BCG Pasteur)	-	WHO, Stockholm
mc ² 2801	BCG Pasteur <i>pcaA</i> ::Tn5370	Hyg	(2)
Plasmids	Description	Marker	Reference
pMV306	<i>attB</i> site specific integrating vector	Km	(3)
pMV306_1351	MSMEG_1351 gene cloned in pMV306	Km	This study
pMV261	Multi-copy plasmid for <i>E.coli</i> and mycobacteria. Cloned genes are under the control of the constitutive <i>hsp60</i> promoter	Km	(3)
pMV261_1351	MSMEG_1351 gene cloned in pMV261	Km	This study
pMV261_1351_1351	<i>pcaA</i> gene cloned in pMV261	Km	(4)

References

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2. Glickman, M. S., Cox, J. S., and Jacobs, W. R., Jr. (2000) *Mol Cell* **5**(4), 717-727
3. Stover, C. K., de la Cruz, V. F., Fuerst, T. R., Burlein, J. E., Benson, L. A., Bennett, L. T., Bansal, G. P., Young, J. F., Lee, M. H., Hatfull, G. F., and et al. (1991) *Nature* **351**(6326), 456-460
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Table S2. Primers used for AES.

Primer name	Sequence	Amplified product
MSMEG0902kontF	GTGATCTTGTTCTTCTTAAGCAGGAAGTGAC GCCCCG	<i>MSMEG0902</i> left arm
MSMEG0902kontR	CAGCGTCATCCCGTCTAGAGGCGAAGTACGC ACAGCTG	
MSMEG0902koctF	CTGATCAGCGTCCCGCTAGCGGGCAGGCGTC CTCGGC	<i>MSMEG0902</i> right arm
MSMEG0902koctR	CCAGCAGACCAAGAAGCTTCTGGTGAGCTACA GCGAC	
MSMEG1203kontF	CAACGTCATCGGGCTTAAGCTGTGCACAATC AGCGC	<i>MSMEG1203</i> left arm
MSMEG1203kontR	AAATACGGCCGCATCTAGACAGTGCCGACGC CGTCAGC	
MSMEG1203koctF	CGGGATCTCCGAGCTAGCACAGTTCGCCTAC ACGAA	<i>MSMEG1203</i> right arm
MSMEG1203koctR	GGCCGGCGCGCTGAAGCTTGTGAGACCGAG GAGCACG	
MSMEG1351kontF	TCGGGGTGATCTTCTTAAGCAACAACCAGGCG CTCCT	<i>MSMEG1351</i> left arm
MSMEG1351kontR	AGGTTCTCGACGGGTCTAGAAACAGCGCGAA GAAGTCGT	
MSMEG1351koctF	GCGTTCGCGTCGGCTAGCTCGACGTCAACC AGTTCAC	<i>MSMEG1351</i> right arm
MSMEG1351koctR	ACCTTCTCGTCGAAAGCTTCCCAGCCCTGCAG GCGG	