

**Table S2. Plasmids used in this study**

<b>Plasmid name</b>	<b>relevant features</b>	<b>relevant details of construction</b>
pMV306hsp+FFluc	<i>attP::P<sub>hsp60</sub>-Ff luciferase kan</i>	Addgene plasmid #26156 [1]
Cerulean	<i>mCerulean</i>	Addgene plasmid #15214 [2]
pDB19	<i>attP::zeo</i>	lab stock
pDB60	<i>attP::strep</i>	lab stock
pDB88	<i>hyg, galK, sacB</i>	lab stock [3]
pMV306kan	<i>attP::kan</i>	lab stock
pMV261kan	<i>P<sub>hsp60</sub> kan oriM</i>	lab stock
pmsg419	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub> hyg oriM</i>	lab stock
pAJF067	<i>hyg, galK, sacB</i>	pDB88 derivative
pAJF198	<i>5'dnaK flank-3' dnaK flank hyg, galK, sacB</i>	pAJF067 with <i>dnaK</i> 5' flank (oAF252/oAF253, MC2155 gDNA) <i>dnaK</i> 3' flank (oAF254/oAF255, MC2155 gDNA)
pAJF200	<i>attP:: dnaK-mCitrine kan</i>	pMV306kan derivative with <i>dnaK</i> (oAF250/251, MC2155 gDNA) and <i>mCitrine</i> (oAF022/oAF023)
pAJF223	<i>attP:: dnaK strep</i>	pDB60 derivative with <i>dnaK</i> (oAF250/266, MC2155 gDNA)
pAJF239	<i>attP:: dnaK(K70A)-mCitrine kan</i>	pAJF200 with 5' <i>dnaK</i> (K70A) (flanks of oAF250/oAF301 and oAF276/oAF302 in pAJF200)
pAJF241	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-dnaK-STII hyg oriM</i>	pmsg419 with <i>dnaK</i> -streptagII (oAF305/oAF280, MC2155 gDNA)
pAJF251	<i>attP::P<sub>ftsZ(P1)</sub>-Ff luciferase kan</i>	pMV306-hsp+FFLuc with <i>PftsW</i> (P1) (oAF312/313, MC2155 gDNA)
pAJF273	<i>5'tig flank-3' tig flank hyg, galK, sacB</i>	pAJF067 derivative with <i>tig</i> 5' flank (oAF347/oAF348, MC2155 gDNA) and <i>tig</i> 3' flank (oAF349/oAF350, MC2155 gDNA)
pAJF298	<i>attB:: PftsZp1-luciferase Pmop-tig kan</i>	pAJF251 derivative with <i>tig</i> (oAF336/oAF337, MC2155 gDNA)
pAJF307	<i>pMV306Kan No Integrase</i>	pMV306kan derivative with integrase sequence removed
pAJF324	<i>3'clpB-mCitrine hyg</i>	3' <i>clpB</i> (oAF400/oAF401, MC2155 gDNA)

pAJF325	<i>5'grpE flank-3' grpE flank hyg, galK, sacB</i>	pAJF067 derivative with grpE 5' flank (oAF412/oAF413, MC2155 gDNA) and grpE 3' flank (oAF414/oAF415, MC2155 gDNA)
pAJF327	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-dnaK-STII kan oriM</i>	derivative of pAJF241 encoding kanamycin resistance
pAJF381	<i>attP(tweety)::P<sub>ftsZ(P1)</sub>-Ff luciferase int(tweety) strep</i>	PftsZ1-Ff luciferase fragment from pAJF251 inserted at EcoRV site
pAJF407	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-dnaK-STII kan oriM</i>	pAJF327 derivative with tig (oAF575/oAF576, MC2155 gDNA)
pAJF417	<i>attP::P<sub>ftsZ(P1)</sub>-Ff luciferase-linker-mCitrine strep</i>	pDB60 derivative with PftsZ(P1)-Ff luciferase from oAJF251 fused to mCitrine
pAJF438	<i>3'fas-linker-mCitrine-STII hyg</i>	3' fasI (oAF642/oAF643, MC2155 gDNA)
pAJF446	<i>P<sub>hsp60</sub>-Ec maL<sub>(TM1,2)</sub>-mCerulean-linker-mCerulean-linker-mCerulean kan</i>	pMV261kan derivative with E. coli maIF <sub>(TM1,2)</sub> (oAF578/579, MG1665 gDNA)
pAJF447	<i>attP:: dnaK-twinStrep kan</i>	pAJF200 with mCitrine removed and twinStrep added by oligo ligation (oAF651/oAF652)
pAJF458	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-dnaK-STII hyg oriM Phsp60-EcmalF1,2-3xmCerulean</i>	pAJF241 derivative with Phsp60-EcmalF1,2-3xmCerulean cut from pAJF446
pAJF466	<i>attB::BCG dnaK-mCitrine strep</i>	BCG dnaK (oAF674/oAF676, BCG gDNA )
pAJF480	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc-tetO</sub>-mCitrine-ELK16 hyg oriM</i>	pmsg419 derivative with mCitrine-ELK16 (tag added with ligation of annealed oligos oAF708 and oAF709)
pAJF488	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-mCerulean-ELK16 hyg oriM</i>	pmsg419 derivative with mCerulean-ELK16 (tag added with ligation of annealed oligos oAF708 and oAF709)
pAJF494	<i>P<sub>Tb21</sub>-tetR(sB) P<sub>myc1-tetO</sub>-mCerulean-ELK16 strep oriM</i>	pAJF488 derivative encoding streptomycin resistance
pAJF499	<i>P<sub>Tb21</sub>-tetR(sB), P<sub>myc1-tetO</sub>-grpE-STII, hyg, oriM</i>	pmsg419 derivative with grpE-STII (oAF469/470, MC2155 gDNA)
pAJF509	<i>attP::P<sub>dnaK</sub>-grpE kan</i>	pMV306kan derivative with PdnaK (oAF330/oAF331, MC2155 gDNA) and grpE (oAF762/764, MC2155 gDNA)
pAJF512	<i>attP::P<sub>dnaK</sub>-grpE strep</i>	pDB60 with PdnaK-grpE from pAJF509

1. Andreu N, Zelmer A, Fletcher T, Elkington PT, Ward TH, et al. (2010) Optimisation of bioluminescent reporters for use with mycobacteria. PLoS One 5: e10777.

- 2 Rizzo MA, Springer GH, Granada B, Piston DW (2004) An improved cyan fluorescent protein variant useful for FRET. *Nat Biotechnol* 22: 445-449.
3. Barkan D, Stallings CL, Glickman MS (2011) An improved counterselectable marker system for mycobacterial recombination using galk and 2-deoxy-galactose. *Gene* 470: 31-36.