The unexpected essentiality of *glnA2* in *Mycobacterium smegmatis* is salvaged by overexpression of the global nitrogen regulator *glnR*, but not by L- or D-glutamine.

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Plasmids used in this study:

Name	Genes	Selection	Origin	Use
pDB221	*glnA2*^{mut}	kanamycin	Attp: integrating	Creating merodiploid mutant for *glnA2*.
pDB240	*glnA2* flanking regions, *sacB, galK*	zeocin	No mycobacterial origin	Deletion of *glnA2*
pBRLUX13	*LUX A-E*	kanamycin	Attp: integrating	Creating a luminescent mutant
pDB234	Empty vector	Zeocin and kanamycin	Attp: integrating	
pDB247	pDB234+*wt_glnA2*			
pDB259	pDB234+*Rv2222c*			
pDB294	pDB234+*Rv1878*			
pDB295	pDB234+*Rv2220c*			
pDB299	*lacZ*	zeocin	Attp: integrating	
pDB328	pDB299+ *wt_glnA2*			
pDB329	pDB299+*Rv2222c*			
pDB316	pDB234+*sacB* under *wt glnR* promoter			
pDB317	pDB234+*sacB* under mutant *glnR* promoter			
pDB342	*glnR* with a mutated promoter	hygromycin	*oriM* (episomal)	To examine the effect of *glnR* overexpression

Strains and mutants used in this study:

Name of strain/mutant	Genotype	Description/phenotype
M. smegmatis wt (mc^2-155)		Reference laboratory strain
mDB67	*M. smeg ΔglnA2; attp:glnA2*^{I133Fmut}: kanamycin	Temperature sensitive
mDB76	mDB67+pDB234. *M. smeg ΔglnA2*;attp:zeocin Spontaneous *glnR* promoter mutation	Full *glnA2* deletion mutant, with mutation in *glnR* promoter. Not temperature sensitive.
mDB153	Wt + pDB316	Used to compare *glnR* promoter activity, wt versus mutated, by RT-PCR.
mDB154	Wt + pDB317	
mDB167	mDB67 + pDB342	Similar to mDB67, but overexpressing *glnR*
mDB149	Wt + pBRLUX13	luminescent wt *M. smegmatis*