

Revised Table S1. Gene quantification with droplet digital PCR and amplicon sequencing. PCR primer pairs used in the study

Target gene	Name	Used in	Sequence (5' – 3')	Product length	Master mix (per reaction)	PCR conditions	Reference (from main article)	
Mercuric reductase (<i>merA</i>)	qmerA1 F	ddPCR	CAT GAC GGT GCA GGA ACT G	101 bp	QX200 MM (2x) 10 μM FW/RV primers Template Total	11 μL 8.33 μL (ea.) 0.33 μL 2 μL 22 μL	40x {95°C 5 min 60°C 1 min} 4°C 5 min 90°C 5 min 10°C ∞	[53]
	qmerA1 R		GCT GCT TCA CAT CCT TGT TG					
	NifF	Nested PCR (long product)	CCA TCG GCG GCA CYT GCG TYA A	1247 bp	EconoTaq PLUS MM (2x) 10 μM FW/RV primers Template Total	25 μL 19 μL (ea.) 2.5 μL 1 μL 50 μL	94°C 2 min 25x {94°C 30s, 61°C 30s, 72°C 90s} 72°C 5 min 10°C ∞	[56]
	NifR		CGC YGC RAG CTT YAA YCY YTC RRC CAT YGT					
	NsfF	Nested PCR (short product)	ATC CGC AAG TNG CVA CBG TNG G	308 bp	EconoTaq PLUS MM (2x) 10 μM FW/RV primers Template from NifF-NifR PCR Total	12.5 μL 9 μL (ea.) 1.25 μL 1 μL 25 μL	94°C 2 min 35x {94°C 30s, 61°C 30s, 72°C 30s} 72°C 5 min 10°C ∞	[56]
	NsfR		CGC YGC RAG CTT YAA YCY YTC RRC CAT YGT (same as NifR)					
	NsfF_CS1	Illumina MiSeq sequencing (adapters in red)	ACA CTG ACG ACA TGG TTC TAC AAT CCG CAA GTN GCV ACB GTN GG	352 bp	(Similar to NsfF – NsfR above)			[56] Common sequence 1 or 2 tags added as required by Illumina MiSeq
	NifR_CS2	(adapters in red)	TAC GGT AGC AGA GAC TTG GTC TCG CYG CRA GCT TYA AYC YYT CRR CCA TYG T					
Glutamate synthetase (<i>glnA</i>)	GS1β	ddPCR	GAT GCC GCC GAT GTA GTA	153-156 bp	QX200 MM (2x) H ₂ O	11 μL 8.33 μL	95°C 5 min 40x {95°C 30 s, 60°C 1 min}	[54]

	GS2γ		AAG ACC GCG ACC TTY ATG CC		10 μM FW/RV primers 1:10 diluted template Total	(ea.) 0.33 μL 2 μL 22 μL	4°C 5 min 90°C 5 min 10°C ∞	
Ribosomal polymerase subunit B (<i>rpoB</i>)	rpoB_ssF	PCR	CDG AAG GYC CRA ACA TYG	375 bp	EconoTaq PLUS MM (2x) H ₂ O 10 μM FW/RV primers Template Total	12.5 μL 9 μL (ea.) 1.25 μL 1 μL 25 μL	94°C 2 min 35x {94°C 30s, 53°C 30s, 72°C 30s} 72°C 5 min 10°C ∞	(This study)
	rpoB_ssR		CYT GRC GYT GCA TGT TRG					
	rpoB_ssF_CS1	Illumina MiSeq sequencing	ACA CTG ACG ACA TGG TTC TAC ACD GAA GGY CCR AAC ATY G	419 bp	(Similar to rpoB_ssF – rpoB_ssR above)			(This study) Common sequence 1 or 2 tags added as required by Illumina MiSeq
	rpoB_ssR_CS2		TAC GGT AGC AGA GAC TTG GTC TCY TGR CGY TGC ATG TTR G					