Table A1. Alignment of the primer sites targeted by the nifHF/nifHR primer pair.

Organism	Accession	Forward priming site (5'-3')		Reverse priming site (5'-3')[a]		Total mismatches	qPCR Efficiency
		primer nifHF		primer nifHR			
		AAAGGYGGWATCGGYAARTCCACCAC	**Mismatches**	**ATGATGGCSATGTAYGCSGCSAACAA**[b]	**Mismatches**		
Frankia sp.	NC_008278[c]	AA**G**GGTGGTATCGGCAAGTCCACCAC	1	ATGATGGCGATGTACGCGGCGAACAA	0	1	1.73
Cyanothece sp.	NC_011884	AAAGGCGG**G**AT**T**GGTAAATCCACCAC	2	ATGATGGCCATGTACGC**T**GCCAACAA	1	3	1.76
N. commune	L23514[c]	AAAGGCGGTATCGGTAAATC**T**ACCAC	1	ATGATGGCGATGTATGC**T**GC**T**AACAA	2	3	1.77
N. punctiforme	NC_010628	AA**G**GGCGGTATCGGTAAATC**T**ACCAC	2	ATGATGGCGATGTTTGC**A**GC**T**AACAA	2	4	1.76
Nostoc sp.	NC_003272	AAAGGCGGTATCGGTAAATC**T**ACCAC	1	ATGATGGCGATGTATGC**T**GC**T**AACAA	2	3	1.77
P. sabinae	HM583800	AAAGGAGGAATCGGCAAATCCACCAC	0	ATGATGGCCATGTACGC**A**GCCAACAA	1	1	1.76
A. brasilense	X51500[c]	AA**G**GGCGGTATCGGCAAGTCCACCAC	1	ATGATGGCG**C**TCTACGCCGCCAACAA	1	2	1.77
B. japonicum	NC_004463	AA**G**GGCGGAATCGGCAAGTCCACCAC	1	ATGATGGC**A**ATGTATGCCGC**A**AACAA	2	3	1.76
M. trichosporium	NZ_ADVE01000002	AA**G**GGCGGTAT**T**GGCAAGTC**G**AC**G**AC	4	ATGATGGCCATGTATGCCGGCAACAA	0	4	n.d.
R. leguminosarum	NC_008381	AA**G**GGCGG**C**AT**T**GGCAAGTCCAC**T**AC	4	ATGATGGCG**C**TCTATGCCGCCAACAA	1	5	1.75
S. meliloti	NC_003037	AA**G**GG**G**GGTATCGGCAAGTCCAC**G**AC	3	ATGATGGCG**C**TCTATGCCGCCAACAA	1	4	1.75
S. azotifigens	AB217474[c]	~~~~~TGGTAT**T**GGTAAGT**GG**AC**G**AC[e]	4	ATGATGGCGATGTATGCCGCCAACAA	0	4	n.d.
X. autotrophicus	NC_009720	AA**G**GGTGG**C**ATCGGCAAGTCCACCAC	2	ATGATGGCCATGTATGCGGCCAACAA	0	2	1.73
A. ferrooxidans	NC_011761	AA**G**GGGGG**C**AT**T**GGCAAGTC**T**ACCAC	4	ATGATGGCCATGTATGCCGCCAACAA	0	4	1.75
B. xenovorans	NC_007952	AAAGGTGG**C**ATCGGCAAGTC**G**ACCAC	2	ATGATGGCCATGTACGCGGCCAACAA	0	2	1.74
G. sulfurreducens	NC_002939	AAAGGCGG**C**ATCGGCAAATC**G**ACCAC	2	ATGATGGCCATGTACGC**T**GCCAACAA	1	3	1.67
A. vinelandii	M20568[c]	AAAGGTGGTATCGGTAAGTCCACCAC	0	ATGATGGCCATGTACGCCGCCAACAA	0	0	1.77
M. capsulatus	NC_002977	AAAGGCGG**C**ATCGGCAAGTCCACCAC	1	ATGATGGCCATGTATGCGGCCAACAA	0	1	1.77
P. stutzeri	NC_009434	AA**G**GGTGGAATCGGCAAATCCACCAC	1	ATGATGGCGATGTATGCCGCCAACAA	0	1	1.71

[a] DNA sequences in the reverse priming site are given in the same sense as in the forward priming site.

[b] Reverse complement to primer nifHR, in order to match the sequence alignment.

[c] Sequences of strains that are closely related to the strains used in the analysis.

[e] Available sequence information on *nifH* of *S. azotifigens* was limited and did not cover the entire priming site.

n.d. - no amplification was detected.

Table A2. Variation of the SC method parameters for dilution series.

independently prepared by five researchers (A–E) and run by one researcher in

one qPCR assay per primer pair.

Template	Target gene primer pair	Dilution series[a]	Slope	Intercept	R^2	n[b]	E_{ds}
B. japonicum	*nifH* nifHF/nifHR	A	-3.946	36.33	0.987	11	1.76
		B	-4.106	36.81	0.988	11	1.79
		C	-4.356	37.95	0.985	14	1.75
		D	-4.188	37.25	0.991	13	1.76
		E	-3.970	36.22	0.981	15	1.77
P. stutzeri	*nifH* nifHF/nifHR	A	-4.732	39.71	0.981	11	1.63
		B	-4.529	39.69	0.964	8	1.66
		C	-4.852	41.06	0.940	9	1.61
		D	-4.983	40.97	0.989	12	1.59
		E	-5.065	40.88	0.984	12	1.58
B. japonicum	16S rRNA 27F/518R	A	-5.000	34.98	0.987	8	1.58
		B	-4.882	43.29	0.986	9	1.60
		C	-5.307	45.18	0.996	9	1.54
		D	-4.835	43.28	0.996	9	1.61
		E	-4.708	42.62	0.995	8	1.63
P. stutzeri	16S rRNA 27F/518R	A	-3.909	32.55	0.99	9	1.80
		B	-3.894	32.40	0.992	10	1.81
		C	-4.077	33.28	0.987	11	1.76
		D	-3.880	32.46	0.995	11	1.81
		E	-3.817	32.19	0.997	12	1.83

[a] five researchers (A-E) independently prepared dilution series of each template.
[b] number of points in calibration.

Figure A1. Variability of qPCR efficiency estimations by dilution series (E_{ds}) and fluorescence increase (E_{fi}). E_{ds} and E_{fi}, and means of E_{ds} and E_{fi} determined on dilution series independently prepared by five researchers (A–E) using the same DNA stock and lab equipment. Error bars indicate the standard deviation. Asterisk indicates significant differences between mean E_{ds} and mean E_{fi} by the Mann-Whitney rank sum test (p<0.01).