

Supplementary Materials: DNA and RNA Extraction and Quantitative Real-Time PCR-Based Assays for Biogas Biocenoses in an Interlaboratory Comparison

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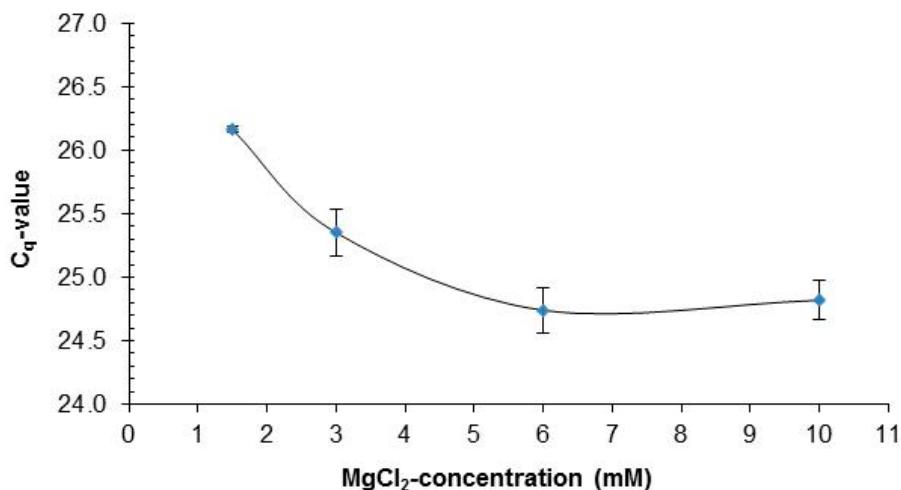


Figure S1. Effect of different MgCl₂ concentrations on the qPCR efficacy. DNA from cattle manure spiked with *E. coli* was extracted using system FSKS and quantified using the *murA* system (see Section 2.3).

Table S1. qPCR assays used in the interlaboratory comparison.

Partner/Analyst	A	B	C	D
DNA quantification	NanoDrop 1000 + PicoGreen	IMPLEN NanoPhotometer™	NanoDrop 1000	NanoDrop 1000 + Picogreen
qPCR reagents	Thermo Scientific DyNAmo Flash Probe qPCR Kit; Mg ²⁺ as supplied by manufacturer;	Self-made master-mix containing 2xEvaGreen, 200 μM dNTPs and 0.75 U Platinum Taq-Polymerase; Mg ²⁺ 6 mM in qPCR assays (Lebuhn <i>et al.</i> , 2003; Munk <i>et al.</i> , 2010);	Biorad SsoAdvanced™ Universal SYBR® Green Supermix; Mg ²⁺ as supplied by manufacturer;	LC480 Roche Probes Master; Mg ²⁺ 3.2 mM in qPCR assays;
Reaction volume	18 μL master-mix + 2 μL extract	24 μL master-mix + 1 μL extract	15 μL master-mix + 2 μL extract	15 μL master-mix + 5 μL extract
Primers and probes	Bac fw Bac probe Bac rev Arc fw Arc Probe Arc rev mcrA/mrtA fw mcrA/mrtA rev	Bac Fw (ACT CCT ACG GGA GGCAG), [1] Bac Probe (6-FAM-TGCCA GCAGC CGCGG TAATA C-TAMRA), [1] Bac-rev (GAC TAC CAG GGT ATC TAA TCC), [1] Arc-fw (ATTAGA TACCCS BGTAGT CC), [1] Arc-Probe (6-FAM-AGGAA TTGGC GGGGG AGCAC-TAMRA), [1] Arc-rev (GCCATG CACCWC CTCT), [1] MeA-i 1046f (TAYATGWSIGGHGGIGTIGGI TTYAC), [2]	Eub 338 (ACTCCTACGGGAGGCAG), [3] Eub 518 (ATTACCGCGGCTGCTGG), [3] Bac-rev (GACTA CCAGG GTATC TAATC C), [1]	Bac Fw (ACTCCTACGGGAGGCAG), [1] Bac Probe (6-FAM-TGCCA GCAGC CGCGG TAATA C-TAMRA), [1] Bac-rev (GACTA CCAGG GTATC TAATC C), [1]
qPCR platform	CFX96 Touch (Biorad)	MX 3005P qPCR System (Agilent)	CFX96 Touch (Biorad)	LightCycler480 (Roche)

Table S1. *Cont.*

Partner/Analyst		A	B	C	D
Protocols	Bac protocols (i, iv, v)	95 °C, 07:00 95 °C, 00:15 57 °C, 00:30 60 °C, 00:60 45x		98 °C, 02:00 98 °C, 00:05 56 °C, 00:35 40x	Pre-Incubation 95 °C, 10:00 95 °C, 00:10 60 °C, 00:30 72 °C, 00:01 (acquisition) 45x
Arc	protocol (ii)	95 °C, 07:00 95 °C, 00:15 60 °C, 00:60 45x			
mcrA/mrtA	protocol (iii)		95 °C, 03:00 95 °C, 00:15 60 °C, 01:00 82 °C, 00:10 (acquisition) 45x		

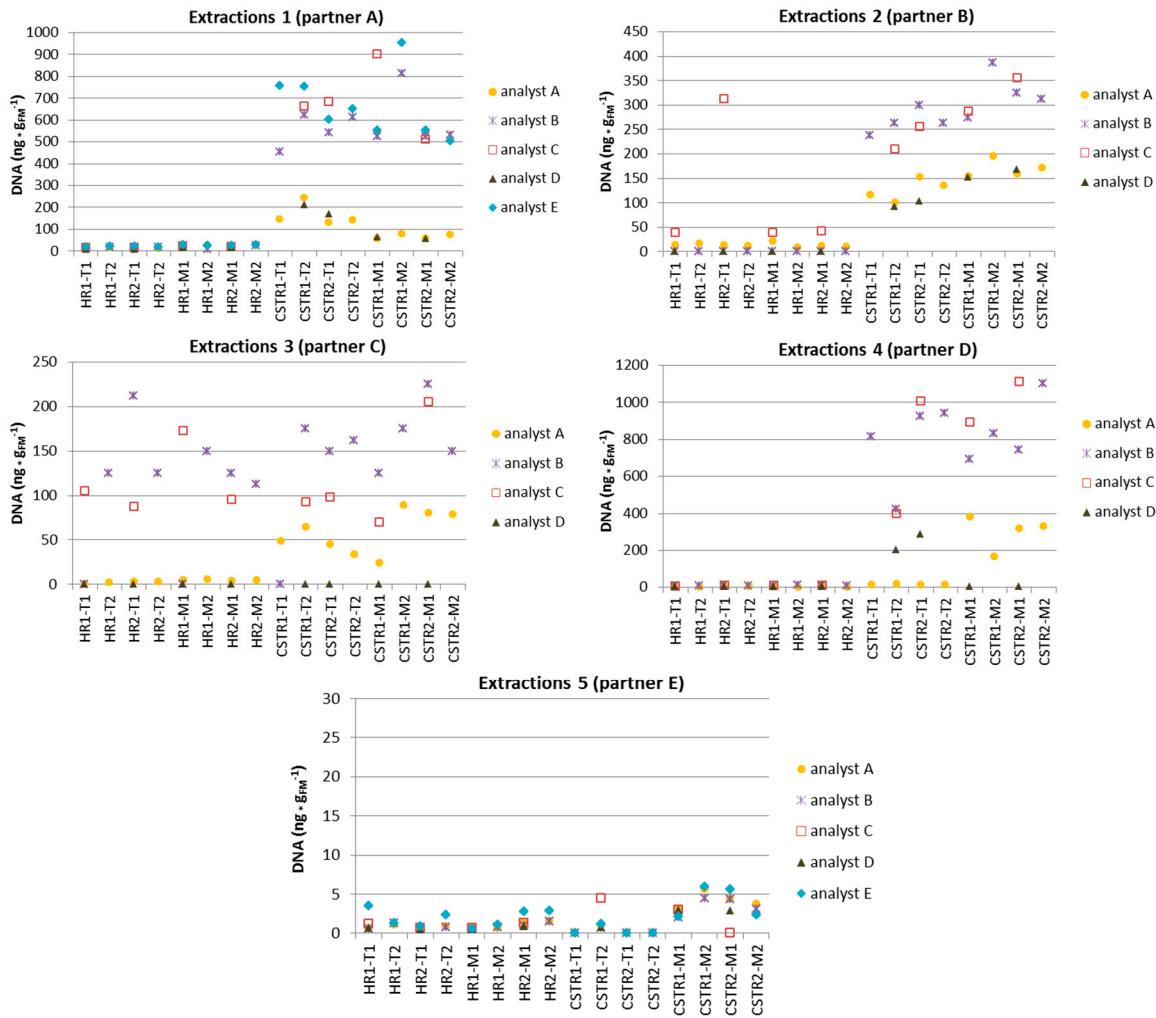


Figure S2. DNA concentrations calculated per mL or g of fresh sample matter of extracts produced by the five partners of the interlaboratory comparison as determined with the extinction coefficient for ds-DNA (analysts B, C and E) or the PicoGreen® system (analysts A, D).

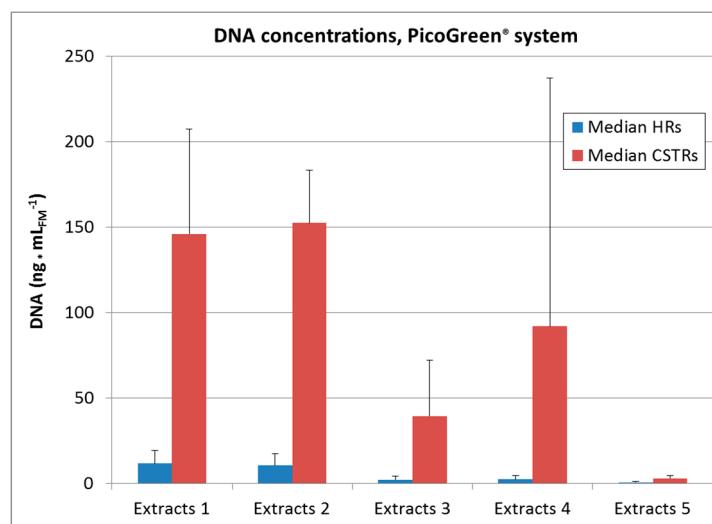


Figure S3. DNA concentrations in the extracts prepared by the partners calculated per mL g of fresh sample matter using only the data from the PicoGreen® system. The medians over the different analysts with the SDs are presented.

Table S2. Absorbance ratios A260/280 and A260/230, and absorbance at 320 nm of extracts produced by the five and analyzed by two partners/analysts of the interlaboratory comparison.

Extracts 1 (Partner A)						Extracts 2 (Partner B)			Extracts 3 (Partner C)				
Analyst B	A260/280	A260/230	A320	Analyst E	A260/280	A260/230	Analyst B	A260/280	A260/230	Analyst B	A260/280	A260/230	A320
HR1-T1	1.767	0.03	0.011	HR1-T1	1.86	0.03	HR1-T1	<d.l.	<d.l.	HR1-T1	2	0.022	0.002
HR1-T2	1.714	0.031	0.014	HR1-T2	1.5	0.04	HR1-T2	<d.l.	<d.l.	HR1-T2	1.667	0.024	0.003
HR2-T1	1.719	0.03	0.014	HR2-T1	1.57	0.04	HR2-T1	<d.l.	<d.l.	HR2-T1	1.7	0.037	0.003
HR2-T2	1.647	0.042	0.014	HR2-T2	1.63	0.05	HR2-T2	<d.l.	<d.l.	HR2-T2	2	0.034	0.004
HR1-M1	1.686	0.042	0.021	HR1-M1	1.54	0.04	HR1-M1	<d.l.	<d.l.	HR1-M1	1.4	0.025	0.003
HR1-M2	2.75	0.044	-0.002	HR1-M2	1.42	0.09	HR1-M2	<d.l.	<d.l.	HR1-M2	2.4	0.017	0.002
HR2-M1	1.756	0.044	0.015	HR2-M1	1.63	0.05	HR2-M1	<d.l.	<d.l.	HR2-M1	1.667	0.032	0.002
HR2-M2	1.826	0.04	0.024	HR2-M2	1.62	0.04	HR2-M2	<d.l.	<d.l.	HR2-M2	1.8	0.033	0.002
CSTR1-T1	1.957	0.021	0.012	CSTR1-T1	1.67	0.02	CSTR1-T1	1.727	0.052	CSTR1-T1	0.8	0.016	0.002
CSTR1-T2	1.676	0.032	0.044	CSTR1-T2	1.67	0.03	CSTR1-T2	1.615	0.041	CSTR1-T2	1.556	0.037	0.003
CSTR2-T1	1.742	0.031	0.015	CSTR2-T1	1.5	0.03	CSTR2-T1	1.6	0.045	CSTR2-T1	2	0.031	0.003
CSTR2-T2	1.848	0.031	0.015	CSTR2-T2	1.63	0.03	CSTR2-T2	1.5	0.146	CSTR2-T2	1.625	0.038	0.002
CSTR1-M1	1.793	0.025	0.014	CSTR1-M1	1.38	0.02	CSTR1-M1	1.692	0.14	CSTR1-M1	1.667	0.033	0.001
CSTR1-M2 *	1.929	0.036	0.028	CSTR1-M2	1.73	0.02	CSTR1-M2	1.632	0.091	CSTR1-M2	1.75	0.044	0.002
CSTR2-M1	1.926	0.028	0.019	CSTR2-M1	1.38	0.03	CSTR2-M1	1.733	0.058	CSTR2-M1	1.8	0.1	0.002
CSTR2-M2	1.71	0.028	0.017	CSTR2-M2	1.67	0.02	CSTR2-M2	1.786	0.139	CSTR2-M2	1.714	0.041	0.001
Extracts 4 (Partner D)						Extracts 5 (Partner E)							
Analyst B	A260/280	A260/230	A320	Analyst B	A260/280	A260/230	A320	Analyst E	A260/280	A260/230			
HR1-T1	1.75	0.019	0.008	HR1-T1	1.765	0.435	0.002	HR1-T1	1.85	1.26			
HR1-T2	1.647	0.019	0.008	HR1-T2	1.806	0.699	0.002	HR1-T2	1.76	0.79			
HR2-T1	1.833	0.019	0.009	HR2-T1	1.933	0.426	0.001	HR2-T1	1.74	0.66			
HR2-T2	1.5	0.061	0.01	HR2-T2	1.905	0.506	0.002	HR2-T2	1.77	1.05			
HR1-M1	1.8	0.017	0.007	HR1-M1	1.812	0.403	0.001	HR1-M1	1.78	0.51			
HR1-M2	1.684	0.022	0.01	HR1-M2	1.818	0.525	0.001	HR1-M2	1.77	0.67			
HR2-M1	1.591	0.031	0.01	HR2-M1	1.759	0.646	0.001	HR2-M1	1.8	1.24			
HR2-M2	1.706	0.022	0.008	HR2-M2	1.795	0.814	0.004	HR2-M2	1.82	1.17			
CSTR1-T1	2.077	0.036	0.016	CSTR1-T1	n.d.	n.d.	n.d.	CSTR1-T1	1.345	0.34			
CSTR1-T2	1.909	0.023	0.008	CSTR1-T2	1.783	1.019	0.004	CSTR1-T2	2.5	1.12			
CSTR2-T1	2.14	0.042	0.021	CSTR2-T1	n.d.	n.d.	n.d.	CSTR2-T1	n.d.	n.d.			
CSTR2-T2	2.293	<d.l.	0.019	CSTR2-T2	n.d.	n.d.	n.d.	CSTR2-T2	n.d.	n.d.			
CSTR1-M1*	2.654	0.03	0.017	CSTR1-M1	1.763	1.305	0.005	CSTR1-M1	2.04	1.34			
CSTR1-M2	2.306	0.036	0.025	CSTR1-M2	1.773	1.376	0.005	CSTR1-M2	1.92	1.89			
CSTR2-M1*	2.552	0.033	0.019	CSTR2-M1	1.775	1.153	0.004	CSTR2-M1	1.93	1.79			
CSTR2-M2*	3.235	<d.l.	0.022	CSTR2-M2	1.8	0.462	0.002	CSTR2-M2	2.06	1.41			

*: qPCR inhibition by undiluted extract; n.d.: not determined/not delivered; <d.l.: below detection limit; red color denotes values below and pink color values above conventional thresholds.

References

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