Supplemental Information

Evaluation of the repeatability and reproducibility of a suite of qPCR-based microbial source tracking methods

Darcy L. Ebentier, Kaitlyn T. Hanley, Yiping Cao, Brian D. Badgley, Alexandria B. Boehm, Jared S. Ervin, Kelly D. Goodwin, Michèle Gourmelon, John F. Griffith, Patricia A. Holden, Catherine A. Kelty, Solen Lozach, Charles McGee, Lindsay A. Peed, Meredith Raith, Hodon Ryu, Michael J. Sadowsky, Elizabeth A. Scott, Jorge Santo Domingo, Alexander Schriewer, Christopher D. Sinigalliano, Orin C. Shanks, Laurie C. Van De Werfhorst, Dan Wang, Stefan Wuertz and Jennifer A. Jay

Table SI-1. Laboratories and Methods Used in this Study

Method Name	Laboratories Doing Analysis	Reference
BacCow	1, 2, 4, 5, 10	Kildare et al. 2007
BacHum	1, 2, 3, 5, 9, 10	Kildare et al. 2007
BSteriF1	1, 2, 4, 5	Haugland et al. 2010
CowM2	1, 2, 3, 4, 5	Shanks et al. 2008
DogBact	3, 4, 5, 7, 9	Sinigalliano et al. 2010
Gull2Taqman	1, 3, 4, 6, 9	Sinigalliano et al. 2010
HF183Taqman	1, 2, 3, 4, 5	Haugland et al. 2010
HumM2	1, 2, 3, 4, 5, 9	Shanks et al. 2009
Pig2Bac	1, 2, 4, 5, 8	Mieszkin et al. 2009
Entero	2, 4, 5	US EPA 2012

Table SI-2. Extraction Methods Used in this Study

Lab No.	Physical Lysis Method	Extraction Kit	Elution Volume
1-5	Mini-Beadbeater-16, 2 min (BioSpec, Bartlesville, OK)	DNA-EZ ST1[1] (Generite, Kendall Park, NJ)	100µl
6	Mini-Beadbeater-8, 40 sec[4] (BioSpec, Bartlesville, OK)	MoBio PowerLyzer PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA)	100µl
7	FastPrep FP120, 2x30s[3] (Qbiogene, Carlsbad, CA)	DNA-EZ ST1[1] (Generite, Kendall Park, NJ)	100µl
8	None.	QIAamp DNA Mini Kit[2](Qiagen, Valencia, CA)	100µl
9	FastPrep FP120, 2x30s[3] (Qbiogene, Carlsbad, CA)	DNA-EZ ST1[1] (Generite, Kendall Park, NJ)	100µl
10	Mini-Beadbeater-8, 1 min[4] (BioSpec, Bartlesville, OK)	DNA-EZ ST1 (Generite, Kendall Park, NJ)	200µl

[1] Utilized modified kit protocol (Boehm et al 2012)

[2] Utilized modified kit protocol (Mieszkin et al 2009)

[3] Speed setting 5.0, 2 rounds of 30 second agitation

[4] Max speed- 2800 oscillations/min

Table SI-3. Standardized Protocols Utilized By Laboratories 1 - 10

Method	Reference	Cycling Conditions			Platform	Sample/ Reaction Vol (ul)	Reaction Recipe		
BacCow Laboratories 1-5	Kildare et al 2005	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50°C	1			ABI Master Mix	2X	12.5
		10 min	95°C				Fwd Primer	10µM	1
		15 sec	95°C	40			Rev Primer	10µM	1
		1 min	60°C				Probe	10µM	0.2
							BSA	75 mg/ml	0.017
							Ultrapure H2O	--	8.28
							Template	Variable	2
BacHum Laboratories 1-5	Kildare et al 2005	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50°C	1			ABI Master Mix	2X	12.5
		10 min	95°C				Fwd Primer	10µM	1
		15 sec	95°C	40			Rev Primer	10µM	1
		1 min	60°C				Probe	10µM	0.2
							BSA	75 mg/ml	0.017
							Ultrapure H2O	--	8.28
							Template	Variable	2
BSteriF1 Laboratories 1-5	Haugland et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50°C	1			ABI Master Mix	2X	12.5
		10 min	95°C				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95°C	40			Primer/Probe Mix	.667 uM probe 8.33 uM Rev primer 33.33 uM Fwd primer	3.5
		1 min	60°C				Ultrapure H2O	--	4.5
							Template	Variable	2
CowM2 Laboratories 1-5	Shanks et al 2008	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50°C	1			ABI Master Mix	2X	12.5
		10 min	95°C				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95°C	40			Primer/Probe Mix	.667 µM probe 8.33 µM Rev primer 8.33 µM Fwd primer	3.5
		1 min	60°C				Ultrapure H2O	--	4.5
							Template	Variable	2

Table SI-3. Continued

Method	Reference	Cycling Conditions			Platform	Sample/ Reaction Vol (ul)	Reaction Recipe		
DogBact Laboratories 1-5	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		15 min	95°C	1			Quantitect Master Mix	2X	12.5
		15 sec	95°C	40			Fwd Primer	10µM	2.25
		30 sec	60°C				Rev Primer	10µM	2.25
							Probe	10µM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2
Gull2Taqman Laboratories 1-5	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		15 min	95°C	1			Quantitect Master Mix	2X	12.5
		15 sec	95°C	40			Fwd Primer	10µM	2.25
		1 min	62°C				Rev Primer	10µM	2.25
							Probe	10µM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2
HF183Taqman Laboratories 1-5	Haugland et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Master Mix	2X	12.5
		10 min	95				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95	40			Primer/Probe Mix	.667 µM probe 8.33 µM Rev primer 8.33 µM Fwd primer	3.5
		1 min	60				Ultrapure H2O	--	4.5
							Template	Variable	2
HumM2 Laboratories 1-5	Shanks et al 2009	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Master Mix	2X	12.5
		10 min	95				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95	40			Primer/Probe Mix	.667 µM probe 8.33 µM Rev primer 8.33 µM Fwd primer	3.5
		1 min	60				Ultrapure H2O	--	4.5
							Template	Variable	2

Table SI-3. Continued

Method	Reference	Cycling Conditions			Platform	Sample/ Reaction Vol (ul)	Reaction Recipe		
Pig2Bac Laboratories 1-5	Mieszkin et al 2009	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Master Mix	2X	12.5
		10 min	95				Fwd Primer	100µM	0.075
		15 sec	95	40			Rev Primer	100µM	0.075
		1 min	60				Probe	100µM	0.05
							Bovine Serum Albumen	2 ng/ml	2.5
							Ultrapure H2O	--	7.8
							Template	Variable	2
Entero Laboratories 1-5	US EPA 2012	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Master Mix	2X	12.5
		10 min	95				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95	40			Primer/Probe Mix	.57 µM probe 7.14 µM Rev primer 7.14 µM Fwd primer	3.5
		1 min	60				Ultrapure H2O	--	4.5
							Template	Variable	2
BacCow Laboratory10	Kildare et al 2005	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus	10*/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Environmental Master Mix	2X	12.5
		10 min	95				Fwd Primer	100µM	0.1
		15 sec	95	40			Rev Primer	100µM	0.1
		1 min	60				Probe	100µM	0.02
							BSA	.962 mg/mL	1.3
							Ultrapure H2O	--	0.98
							Template*	Variable	10
BacHum Laboratory 9	Kildare et al 2005	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			Quantitect Master Mix	2X	12.5
		10 min	95				Fwd Primer	10µM	1
		15 sec	95	40			Rev Primer	10µM	1
		1 min	60				Probe	10µM	0.2
							BSA	75 mg/ml	0.017
							Ultrapure H2O	--	8.28
							Template	Variable	2

Table SI-3. Continued

Method	Reference	Cycling Conditions			Platform	Sample/ Reaction Vol (ul)	Reaction Recipe		
BacHum 10 Laboratory	Kildare et al 2005	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus	10*/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		2 min	50	1			ABI Environmental Master Mix	2X	12.5
		10 min	95				Fwd Primer	100µM	0.1
		15 sec	95	40			Rev Primer	100µM	0.1
		1 min	60				Probe	100µM	0.02
							BSA	.96154 mg/mL	1.3
							Ultrapure H2O	--	0.98
							Template*	Variable	10
DogBact Laboratory 7	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	Stratagene MX3000P	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		15 min	95	1			ABI Master Mix	2X	12.5
		15 sec	95	40			Fwd Primer	10µM	2.25
		30 sec	60				Rev Primer	10µM	2.25
							Probe	10µM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2
DogBact Laboratory 9	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		15 min	95	1			Quantitect Master Mix	2X	12.5
		15 sec	95	40			Fwd Primer	10µM	2.25
		30 sec	60				Rev Primer	10µM	2.25
							Probe	10µM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2
Gull2Taqman Laboratory 6	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	ABI 7900 Fast	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		10 min	95	1			ABI Master Mix	2X	12.5
		15 sec	95	45			Fwd Primer	10uM	2.25
		1 min	60				Rev Primer	10uM	2.25
							Probe	10uM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2
Gull2Taqman Laboratory 9	Sinigalliano et al 2010	*Time*	*Temp*	*No. Cycles*	ABI StepOnePlus	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		15 min	95	1			Quantitect Master Mix	2X	12.5
		15 sec	95	40			Fwd Primer	10µM	2.25
		1 min	62				Rev Primer	10µM	2.25
							Probe	10µM	0.75
							Ultrapure H2O	--	5.25
							Template	Variable	2

Table SI-3. Continued

Method	Reference	Cycling Conditions			Platform	Sample/ Reaction Vol (ul)	Reaction Recipe		
		Time	*Temp*	*No. Cycles*			*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
HumM2 Laboratory 9	Shanks et al 2009	2 min	50	1	ABI StepOnePlus	2/25	Quantitect Master Mix	2X	12.5
		10 min	95				Bovine Serum Albumen	2 mg/ml	2.5
		15 sec	95	40			Primer/Probe Mix	.667 µM probe 8.33 µM Rev primer 8.33 µM Fwd primer	3.5
		1 min	60				Ultrapure H2O	--	4.5
							Template	Variable	2
Pig2Bac Laboratory 8	Mieszkin et al 2009	*Time*	*Temp*	*No. Cycles*	Bio-Rad CFX-96	2/25	*Reagent*	*Stock Sol*	*Per Rxn Vol (µl)*
		10 min	95	1			Platinum Super Mix-UDG	2X	12.5
		15 sec	95	40			Fwd Primer	20µM	0.375
		1 min	60				Rev Primer	20µM	0.375
							Probe	5µM	1
							Ultrapure H2O	--	8.5
							Template	Variable	2

* Laboratory 10 analyzed multiple dilutions of sample DNA. Template + diluent (Ultrapure H2O) volume was always 10µl

Table SI-4. Reagent Sources for Laboratories 1 - 5

Item	Product No.	Source	Specifications
Primers	NA	Operon	Salt Free
Taqman Probes	NA	Operon	HPLC Purified
Universal Master Mix	4318157	ABI	
QuantiTect Master Mix	204435	Qiagen	
Bovine Serum Albumen	15260-037	Invitrogen	
DNAse free water	17012-5200	MO BIO	
AE Buffer	19077	Qiagen	

Table SI-5. DNA Reference Standards for Laboratories 1-5

Method Name	Standard Source	Extracted/ Prepared By	Notes
BacCow	Wuertz Lab, University of California, Davis	Stanford	Circular plasmid
BacHum	Wuertz Lab, University of California, Davis	Stanford	Circular plasmid
BSteriF1	IDT Technologies	SCCWRP	Linearized plasmid
CowM2	IDT Technologies	SCCWRP	Linearized plasmid
DogBact	Dr. Sinigalliano, NOAA	SCCWRP	Circular plasmid
Gull2Taqman	Dr. Sinigalliano, NOAA	SCCWRP	Circular plasmid
HF183Taqman	IDT Technologies	SCCWRP	Linearized plasmid
HumM2	IDT Technologies	SCCWRP	Linearized plasmid
Pig2Bac	IDT Technologies	SCCWRP	Linearized plasmid
Entero	ATCC® *E. faecalis* #29212™	UCLA	Extracted/purified genomic DNA