Supplementary Information

Table S1. The composition of the metagenomic control material (MCM) ([15]).

Bacteria	ATCC No.	Genome Size (MB)	Mol. Wt. (Da)	ng per µL (1×)	% Gram Weight	Genomic Copy Number per µL (1×)	% Genomic Copy Number	16S Copies	16S Copies µL (1×)	% 16S	Relative Standard Deviation
MRSA	BAA-1556D	2.872769	1.8960×10^{9}	4.00×10^{-3}	0.80%	1.27×10^{3}	0.75%	5	6.35×10^{3}	0.78%	4.00%
MSSA	BAA-1718D-5	2.872915	1.8549×10^{9}	6.00×10^{-2}	12.00%	1.95×10^{4}	11.52%	5	9.74×10^{4}	11.96%	5.09%
S. pneumoniae	700669D-5	2.221315	1.4661×10^{9}	1.28×10^{-1}	25.60%	5.26×10^{4}	31.09%	4	2.10×10^{5}	25.83%	2.19%
S. pyogenes	700294D-5	1.852441	1.2226×10^{9}	2.00×10^{-2}	4.00%	9.85×10^{3}	5.83%	6	5.91×10^{4}	7.26%	3.49%
S. agalactiae	BAA-611D-5	2.160267	1.4258×10^{9}	7.50×10^{-3}	1.50%	3.17×10^{3}	1.87%	7	2.22×10^{4}	2.72%	23.84%
E. faecalis	700802D-5	3.34194	2.2057×10^{9}	5.00×10^{-3}	1.00%	1.37×10^{3}	0.81%	4	5.46×10^{3}	0.67%	3.66%
P. aeruginosa	47085D-5	6.264404	4.1345×10^{9}	5.00×10^{-3}	1.00%	7.28×10^{2}	0.43%	4	2.91×10^{3}	0.36%	9.17%
K. pneumoniae	700721D-5	5.31512	3.5080×10^{9}	1.20×10^{-1}	24.00%	2.06×10^{4}	12.18%	8	1.65×10^{5}	20.24%	4.34%
A. baumannii	17978D-5	4.00075	2.6405×10^{9}	5.00×10^{-4}	0.10%	1.14×10^{2}	0.07%	5	5.70×10^{2}	0.07%	20.76%
E. coli	700928D-5	5.231428	3.4527×10^{9}	1.00×10^{-2}	2.00%	1.74×10^{3}	1.03%	7	1.22×10^{4}	1.50%	20.43%
N. meningitidis	700532D-5	2.194961	1.4487×10^{9}	1.40×10^{-1}	28.00%	5.82×10^{4}	34.42%	4	2.33×10^{5}	28.60%	13.65%

Table S2. The estimation of *P. aeruginosa* with and without dsDNA BR reagent using Qubit Fluorometer 2.0 with the Qubit dsDNA BR Assay Kit (Life Technologies).

Condition	*P. aeruginosa* (ng/µL)				TE Buffer (ng/µL)			
	1	2	3	Average	1	2	3	Average
With reagent	58.4	58	58.6	58.33	<0.010	<0.010	<0.010	<0.010
Without reagent	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Table S3. Fold change of the relative composition of the MCM according to the sequencing methods *versus* the dPCR analysis.

Bacteria	Fold Change				
	α	**β**	**γ**	**δ**	**WGS**
A. baumannii	0.79	0.41	1.36	2.03	1.94
P. aeruginosa	0.51	0.77	1.16	1.14	1.58
E. faecalis	2.41	0.54	1.00	1.02	2.04
E. coli	0.61	1.34	0.68	0.80	1.29
S. agalactiae	2.66	0.61	1.13	1.23	1.53
S. pyogenes	2.66	0.58	0.85	0.90	1.75
K. pneumoniae	0.54	1.34	0.79	0.79	1.06
S. aureus	0.55	2.07	1.52	1.65	1.80
S. pneumoniae	2.42	0.65	0.87	0.90	0.94
N. meningitidis	0.73	1.09	1.05	0.97	0.64

Table S6. The SILVA database (Quast *et al.*, 2013 [25]) contains mis-annotations which can lead to incorrect classification of reads, or shift the classification of reads to a higher taxonomic level when a lowest common ancestor analysis is used, as is the case for MEGAN (Huson *et al.*, 2011 [31]). The table illustrates a few such examples of mis-annotated sequences. MEGABLAST hits against the NCBI non-redundant Nucleotide database (accessed 16 May 2013) were used to verify the true identity of the sequences (Morgulis *et al.*, 2008 [30]). The taxonomic description of the BLAST hits are copied directly from the BLAST search results pages (accession numbers in brackets). The BLAST hits against the NCBI database clearly demonstrate that the SILVA taxonomic string for those sequences is incorrect. This is also verified by the phylogenetic tree shown in supplementary figure Y. In some cases (e.g., AB680060.1.1465 and JQ315432.1.1486) the SILVA species description is correct but the rest of the taxonomic string is incorrect. In other cases (e.g., HQ204284.1.1501) the whole taxonomic string is clearly in error.

SILVA Accession Number	SILVA Taxonomic Classification	Putative MCM Species	Top 5 BLAST Hits against NCBI nr nt
CACX01001585.64.1585	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Strongyloides ratti	*Escherichia coli*	Escherichia coli O145:H28 str. RM12581, complete genome (CP007136.1) Escherichia coli O145:H28 str. RM12761, complete genome (CP007133.1) Escherichia coli O145:H28 str. RM13514, complete genome (CP006027.1) Escherichia coli O145:H28 str. RM13516, complete genome (CP006262.1) Escherichia coli Xuzhou21, complete genome (CP001925.1)
AB680060.1.1465	Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium; Klebsiella pneumoniae	*Klebsiella pneumoniae*	Klebsiella sp. PSB-08 16S ribosomal RNA gene, partial sequence (KF761520.1) Klebsiella pneumoniae gene for 16S rRNA, partial sequence, strain: NBRC 3318 (AB680060.1) Uncultured Klebsiella sp. clone SL16 16S ribosomal RNA gene, partial sequence (HQ264076.1) Uncultured Klebsiella sp. clone SL15 16S ribosomal RNA gene, partial sequence (HQ264075.1) Klebsiella pneumoniae strain SY-1 16S ribosomal RNA gene, partial sequence (GU373625.1)

Table S6. *Cont*.

SILVA Accession Number	SILVA Taxonomic Classification	Putative MCM Species	Top 5 BLAST Hits against NCBI nr nt
HQ204286.1.1501	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; Achromobacter; bacterium NN169S	*Klebsiella pneumoniae*	Klebsiella sp. NN169S 16S ribosomal RNA gene, partial sequence (HQ204286.2) Uncultured Klebsiella sp. clone SL17 16S ribosomal RNA gene, partial sequence (HQ264077.1) Uncultured Klebsiella sp. clone SL12 16S ribosomal RNA gene, partial sequence (HQ264072.1) Klebsiella sp. D3S 16S ribosomal RNA gene, partial sequence (GU259534.1) Klebsiella sp. enrichment culture clone M1 16S ribosomal RNA gene, partial sequence (JN036433.1)
HQ204295.1.1454	Bacteria; Actinobacteria; Actinobacteria; Corynebacteriales; Nocardiaceae; Rhodococcus; bacterium YX118S	*Klebsiella pneumoniae*	Klebsiella sp. YX118S 16S ribosomal RNA gene, partial sequence (HQ204295.2) Klebsiella pneumoniae strain 2 16S ribosomal RNA gene, partial sequence (DQ444287.1) Klebsiella pneumoniae strain SV1 16S ribosomal RNA gene, partial sequence (KF906836.1) Klebsiella pneumoniae JM45, complete genome (CP006656.1) Klebsiella pneumoniae strain PB12 16S ribosomal RNA gene, partial sequence (KF192506.1)
HQ204284.1.1501	Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Stenotrophomonas; bacterium LC55S	*Klebsiella pneumoniae*	Klebsiella sp. LC55S 16S ribosomal RNA gene, partial sequence (HQ204284.2) Uncultured Klebsiella sp. clone SL16 16S ribosomal RNA gene, partial sequence (HQ264076.1) Uncultured Klebsiella sp. clone SL09 16S ribosomal RNA gene, partial sequence (HQ264069.1) Klebsiella pneumoniae 342, complete genome (CP000964.1) Klebsiella pneumoniae strain HUB-IV-004 16S ribosomal RNA gene, partial sequence (JN848784.1)

Table S6. *Cont*.

SILVA Accession Number	SILVA Taxonomic Classification	Putative MCM Species	Top 5 BLAST Hits Against NCBI nr nt
JQ315432.1.1486	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Acinetobacter; Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	Pseudomonas aeruginosa strain Y2P7 16S ribosomal RNA gene, partial sequence (EU221383.1) Pseudomonas aeruginosa strain Y2P3 16S ribosomal RNA gene, partial sequence (EU221381.1) Pseudomonas aeruginosa strain S20410 16S ribosomal RNA gene, partial sequence(KF956583.1) Pseudomonas aeruginosa strain Y2P2 16S ribosomal RNA gene, partial sequence (EU221380.1) Pseudomonas aeruginosa PA96 genome (CP007224.1)

Methods—SILVA mis-Annotations Tree

The *Klebsiella pneuomniae* (NC_009648.1)*, Escherichia coli* (NC_004431.1) and *Pseudomonas aeruginosa* (NC_002516.2) 16S rRNA sequences are from the MCM reference strains and were downloaded from the NCBI. The other sequences were extracted from SILVA and verified by MEGABLAST searches against the NCBI non-redundant Nucleotide database (accessed on 16 May 2013); these are SILVA accessions: AB453329.1.1236, AB010951.1.1478, AB010840.1.1318, AB046357.1.1493, AB008509.1.1467 and AB099655.1.1533. The phylogenetic tree was rooted by centrality and constructed using bioNJ (Gascuel, 1997 [37]) based on a Muscle alignment (Edgar, 2004 [38]) performed in Seaview (Galtier *et al*., 1996 [39]).

Table S7. Primers and probes which were used for qPCR and dPCR in this study. Assays targeting *N. meningitidis* and *S. pnuemoniae* were used to determine the stability of the MCM.

Organism	Target	Accession No.	Primer/Probe	Sequence (5'-3')	Amplicon Size (bp)	Reference
N. meningitidis	*ctrA*	AM4210808	CtrA_F	GCCGTTTGTTGGCGATATTT	150	This study
			CtrA_R	GCACGAATCACCGACACATT		
			CtrA_HP	[FAM] CGGTGGTCGGTAAAACGCCTGG [BHQ1]		

Table S7. *Cont.*

Organism	Target	Accession No.	Primer/Probe	Sequence (5'-3')	Amplicon Size (bp)	Reference
S. pnuemoniae	*lytA*	HG531769	LytA-F	ACGCAATCTAGCAGATGAAGC	101	Harris *et al.* [21]
			LytA-R	TGTTTGGTTGGTTATTCGTGC		
			LytA_HP	[FAM] TTTGCCGAAAACGCTTGATACAGGG [BHQ1]		
E. coli	*uidA*	AE014075	uidA_F	GCCCGCTTCGAAACCAAT	120	This study
			uidA_R	TCGCATTACCCTTACGCTGAA		
			uidA_HP	[FAM] TCCATGTTCATCTGCCCAGTCGAGC [BHQ1]		
S. aureus	*coA*	AB436985	coA_F	GTAGATTGGGCAATTACATTTTGGAGG	117	This study
			coA_R	CGCATCTGCTTTGTTATCCCATGTA		
			coA_HP	[FAM] TAGGCGCATTAGCAGTTGCATC [BHQ1]		
S. pyogenes	*csrR*	JX414161	csrR_F	TGGATGTGGTTGCAGGTTTAGAC	79	This study
			csrR_R	CGGGCAAGTAGTTCTTCAATGG		
			csrR_HP	[FAM] CGGTGCAGACGACTATATTGTTAAACC [BHQ1]		
P. aeruginosa	*regA*	EU342000	regA_F	TGCTGGTGGCACAGGACAT	65	Lee *et al.* [40]
			regA_R	TTGTTGGTGCAGTTCCTCATTG		
			regA_MGB	[FAM] CAGATGCTTTGCCTCAA [BHQ1]		
K. pnuemoniae	*Khe*	AF293352	Khe_F	GATGAAACGACCTGATTGCATTC	77	Hartman *et al.* [41]
			Khe_R	CCGGGCTGTCGGGATAAG		
			Khe_HP	[FAM] CGCGAACTGGAAGGGCCCG [BHQ1]		
E. faecalis	*groES*	AF335185	groES_F	TTACTGTGTCACCAATTTTTACTTCCA	96	This study
			groES_R	AACCACAAACAGGTGAAGTTATCG		
			groES_HP	[FAM] TGCCATTTTCAAGCACACGACCTTCA [BHQ1]		
S. agalactiae	*sip*	HQ878436	sip_F	ATCCTGAGACAACACTGACA	78	This study
			sip_R	TTGCTGGTGTTTCTATTTTCA		
			sip_HP	[FAM] ATCAGAAGAGTCATACTGCCACTTC [BHQ1]		

Table S7. *Cont.*

Organism	Target	Accession No.	Primer/Probe	Sequence (5'-3')	Amplicon Size (bp)	Reference
A. baumannii	*ompA*	KJ363323	ompA_1F(G)	CATGGAACTTCGTGTGTTCTTTG	111	This study
			ompA_2F(A)	CATGGAACTTCGTGTATTCTTTG		
			ompA_R	GCAGTAGCGTTAGGGTATTCAGATAAT		
			ompA_MGB	[FAM] AAATCAAACATCAAAGACC [MGB]		

Table S8. Digital MIQE checklist for authors, reviewers and editors.

Item to Check	Importance	Checklist	Comments/Where?
Experimental Design			
Definition of experimental and control Groups	E	N/A	
Number within each group.	E	N/A	
Assay carried out by core lab or investigators' lab?	D	YES	At both core and investigators laboratory
Power analysis	D	N/A	
Sample			
Description	E	N/A	
Volume or mass of sample processed	E	N/A	
Microdissection or macrodissection	E	N/A	
Processing procedure	E	N/A	
If frozen, how and how quickly?	E	N/A	
If fixed, with what, how quickly?	E	N/A	
Sample storage conditions and duration (especially for FFPE samples)	E	N/A	

Table S8. *Cont.*

Item to Check	Importance	Checklist	Comments/Where?
Nucleic Acid Extraction			
Quantification—instrument/method	E	YES	Experimental Section
Storage conditions: Temperature, concentration, duration, buffer	E	YES	Figure S1
DNA or RNA quantification	E	YES	DNA
Quality/integrity—instrument/method; e.g., RIN/RQI and trace or 3':5'	E	YES	Figure S2
Template structural information	E	N/A	
Template modification (digestion, sonication, pre-amplification *etc.*)	E	N/A	
Template treatment (initial heating or chemical denaturation)	E	YES	Experimental Section
Inhibition dilution or spike	E	YES	Each assay was performed on dilutions of the template. All assays displayed a linear relationship between amplification and copy number
DNA contamination assessment of RNA sample	E	N/A	
Details of DNase treatment where performed	E	N/A	
Manufacturer of reagents used and catalogue number	D	N/A	
Storage of nucleic acid: temperature, concentration, duration, buffer	E	YES	Samples were stored in TE Buffer at −80 °C for up to 12 months

Table S8. *Cont.*

Item to Check	Importance	Checklist	Comments/Where?
Reverse Transcription (If Necessary)			
cDNA priming method + concentration	E	N/A	
One or two step protocol	E	N/A	
Amount of RNA used per reaction	E	N/A	
Detailed reaction components and conditions	E	N/A	
RT efficiency	D	N/A	
Estimated copies measured with and without addition of RT *	D	N/A	
Manufacturer of reagents used and catalogue number	D	N/A	
Reaction volume (for two step reverse transcription reaction)	D	N/A	
Storage of cDNA: temperature, concentration, duration, buffer	D	N/A	
dPCR Target Information			
Sequence accession number	E	YES	Table S6
Location of amplicon	D	YES	Available on request
Amplicon length	E	YES	Table S6
In silico specificity screen (BLAST, *etc*.)	E	YES	This was performed using BLAST, all assays were 100 % specific to target
Pseudogenes, retropseudogenes or other homologs?	D	YES	N/A
Sequence alignment	D	YES	Available on request

Table S8. *Cont.*

Item to Check	Importance	Checklist	Comments/Where?
Secondary structure analysis of amplicon and GC content	D	YES	Analysis was performed using Primer Express 3.0. Primers were selected according to the criteria of the program
Location of each primer by exon or intron (if applicable)	E	N/A	
Where appropriate, which splice variants are targeted?	E	N/A	
dPCR Oligonucleotides			
Primer sequences and/or amplicon context sequence **	E	YES	Table S6
RTPrimerDB Identification Number	D	N/A	
Probe sequences **	D	YES	Table S6
Location and identity of any modifications	E	YES	Table S6
Manufacturer of oligonucleotides	D	YES	Primers and dual labelled probes were manufactured by SIGMA and Eurofins. MGB probes were manufactured by Applied Biosystems.
Purification method	D	YES	HPLC
dPCR Protocol			
Complete reaction conditions	E	YES	Experimental Section
Reaction volume and amount of RNA/cDNA/DNA	E	YES	Experimental Section

Table S8. *Cont*.

Item to Check	Importance	Checklist	Comments/Where?
Primer, (probe), Mg++ and dNTP concentrations	E	YES	Experimental Section; Manufacturer's proprietary
Polymerase identity and concentration	E	YES	Experimental Section; concentration is Manufacturer's proprietary
Buffer/kit Catalogue No and manufacturer	E	YES	Experimental Section; Biotium Fast Probe Master Mix with Rox (31016-1), ABI Gene Expression Master Mix (4369016)
Exact chemical constitution of the buffer	D	NO	Manufacturers' proprietary
Additives (SYBR Green I, DMSO, *etc*.)	E	N/A	
Plates/tubes Catalogue No and manufacturer	D	YES	Fluidigm (100-6152)
Complete thermocycling parameters	E	YES	Experimental Section
Reaction setup	D	YES	Experimental Section
Gravimetric or volumetric dilutions (manual/robotic)	D	YES	Volumetric dilutions (manual)
Total PCR reaction volume prepared	D	YES	Experimental Section
Partition number	E	YES	Experimental Section
Individual partition volume	E	YES	Experimental Section
Total volume of the partitions measured (effective reaction size)	E	YES	Experimental Section
Partition volume variance/standard deviation	D	N/A	

Table S8. *Cont*.

Item to Check	Importance	Checklist	Comments/Where?
Comprehensive details and appropriate use of controls	E	YES	Experimental Section and Results
Manufacturer of dPCR instrument	E	YES	Experimental Section
dPCR Validation			
Optimisation data for the assay	D	YES	Available on request
Specificity (when measuring rare mutations, pathogen sequences *etc*.)	E	YES	This was checked using qPCR
Limit of detection of calibration control	D	N/A	
If multiplexing, comparison with singleplex assays	E	N/A	
Data Analysis			
Average copies per partition (λ or equivalent)	E	YES	λ (average) 1.07
dPCR analysis program (source, version)	E	YES	Experimental Section
Outlier identification and disposition	E	N/A	
Results of NTCs	E	YES	All NTCs gave negative results
Examples of positive(s) and negative experimental results as supplemental data	E	YES	Supplementary Table
Where appropriate, justification of number and choice of reference genes	E	YES	
Where appropriate, description of normalisation method	E	YES	Experimental Section
Number and concordance of biological replicates	D	N/A	
Number and stage (RT or qPCR) of technical replicates	E	YES	Experimental Section
Repeatability (intra-assay variation)	E	YES	Figure 7
Reproducibility (inter-assay/user/lab *etc.* variation)	D	N/A	
Experimental variance or confidence interval ***	E	YES	Results

Table S8. *Cont.*

Item to Check	Importance	Checklist	Comments/Where?
Statistical methods used for analysis	E	YES	Experimental Section
Data submission using RDML	D	N/A	

All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if possible. * Assessing the absence of DNA using a no RT assay (or where RT has been inactivated) is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of a no-RT control is desirable, but no longer essential; ** Disclosure of the primer and probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information when it is not available assay context sequences must be submitted (48); *** When single dPCR experiments are performed, the variation due to counting error alone should be calculated from the binomial (or suitable equivalent) distribution.

Table S9. Minimum information about any (x) sequence (MIxS).

Structured Comment Name	Amplicon Sequencing	Whole Genome Sequencing
submitted_to_insdc	N/A	N/A
investigation_type	bacteria	bacteria
project_name	metagenomic control material	metagenomic control material
experimental_factor		
lat_lon	N/A	N/A
depth	N/A	N/A
alt_elev	N/A	N/A
geo_loc_name	N/A	N/A
collection_date	N/A	N/A
env_biome	N/A	N/A
env_feature	N/A	N/A
env_material	N/A	N/A
env_package	N/A	N/A
subspecf_gen_lin		
ploidy	haploid	haploid
num_replicons		

Table S9. *Cont.*

Structured Comment Name	Amplicon Sequencing	Whole Genome Sequencing
extrachrom_elements		
estimated_size	3.5455395 Mbp	3.5455395 Mbp
ref_biomaterial	doi:10.1007/s00769-012-0941-z	doi:10.1007/s00769-012-0941-z
source_mat_id		
pathogenicity	bacteria	bacteria
biotic_relationship	N/A	N/A
specific_host	N/A	N/A
host_spec_range	N/A	N/A
health_disease_stat	N/A	N/A
trophic_level	N/A	N/A
propagation	N/A	N/A
encoded_traits	N/A	N/A
rel_to_oxygen	N/A	N/A
isol_growth_condt	N/A	N/A
samp_collect_device	N/A	N/A
samp_mat_process	N/A	N/A
samp_size	N/A	N/A
nucl_acid_ext	ATCC proprietary information	ATCC proprietary information
nucl_acid_amp	Lib-L method March 2012 version (Roche GS Junior)	N/A
lib_size		
lib_reads_seqd	123528 (mean)	123528 (mean)
lib_const_meth	paired-end	paired-end
lib_vector	N/A	N/A
lib_screen	N/A	N/A
target_gene	16S rRNA	N/A
target_subfragment	V1, 2, 4, 5 and 6	N/A

Table S9. *Cont.*

Structured Comment Name	Amplicon Sequencing	Whole Genome Sequencing
pcr_primers	In figure	N/A
mid	N/A	
adapters	FA'(CCATCTCATCCCTGCGTG TCTCCGACTCAG)-MIDs-515F and FB'(CCTATCCCCTGTGTGCCT TGGCAGTCTCAG)-909R	
pcr_cond	initial denaturation: 94degC_3min; annealing: 94degC_15sec; elongation: 61degC_45sec; final elongation: 72degC_60sec; total cycles 35; extenstion: 72degC_8min	
seq_meth	Pyrosequencing	Illumina
seq_quality_check	Manually edited	Manually edited
chimera_check	ChimeraSlayer	ChimeraSlayer
assembly		
assembly_name		
finishing_strategy		
annot_source		
url		
sop		