

Supplementary Information

List of Tables

S1	Expected Relative Abundance tables for all communities.	S4
S2	Pairwise Wilcoxon tests for AD, Sens, and FPRA. The FDR (Benjamini-Hochberg procedure) corrected p-values are shown in the table. Bold values indicate FDR-BH < 0.05.	S5
S3	Table of WGS2 parameters used.	S5
S4	Confusion matrices for bioBakery3, bioBakery4, JAMS, WGS2, and Woltka at 0% filtering threshold. tp = True Positive, fp = False Positive, fn = False Negative, TN = True Negative, sens = Sensitivity, spec = specificity, prec = Precision, acc = Accuracy, f1 = F1 Score	S8
S5	Confusion matrices for bioBakery3, bioBakery4, JAMS, WGS2, and Woltka at 0.1% filtering threshold. tp = True Positive, fp = False Positive, fn = False Negative, TN = True Negative, sens = Sensitivity, spec = specificity, prec = Precision, acc = Accuracy, f1 = F1 Score	S11
S6	Relative Time per CPU for all NIST samples. If multiple samples were run in parallel, the average was taken. The Woltka time is the sum of both the alignment and classification steps for each sample.	S11
S7	Table of the Number of False Positive species. A perfect score is 0.	S11
S8	Table of Unclassified metrics. The values shown are percentages. A perfect score is 0%. Neither bioBakery nor Woltka give the number of unclassified by default.	S12

List of Figures

S1	Bar Plot graphical representation of confusion matrices shown in Supplemental Table S4.	S13
S2	Bar Plot graphical representation of confusion matrices shown in Supplemental Table S5.	S14

Species	RA (%)	TAXID
<i>Halomonas</i> sp. HL-93	19.49	1666906
<i>Muricauda</i> sp. ES.050	18.03	1798204
<i>Halomonas</i> sp. HL-4	17.43	1761789
<i>Marinobacter</i> sp. LV10R510-8	15.75	1415571
<i>Psychrobacter</i> sp. LV10R520-6	11.11	1415574
<i>Cohaesibacter</i> sp. ES.047	7.07	1798205
<i>Marinobacter</i> sp. LV10MA510-1	4.88	1415567
<i>Thioclava</i> sp. ES.032	3.15	1798206
<i>Propionibacteriaceae bacterium</i> ES.041	1.16	1945889
<i>Micromonospora echinofusca</i> DSM 43913	1.04	47858
<i>Micromonospora echinaurantiaca</i> DSM 43904	0.88	47857
<i>Micromonospora coxensis</i> DSM 45161	0.00448	356852

(a) Expected relative abundance for BMock12.

Species	RA (%)	TAXID
<i>Bordetella pertussis</i>	25.41	520
<i>Bordetella petrii</i>	18.14	94624
<i>Bordetella pseudohinzii</i>	9.73	1331258
<i>Clostridium aceticum</i>	7.50	84022
<i>Bacteroides thetaiotaomicron</i>	6.64	818
<i>Clostridium butyricum</i>	3.24	1492
<i>Bacteroides fragilis</i>	3.10	817
<i>Clostridium botulinum</i>	2.98	1491
<i>Clostridium pasteurianum</i>	2.65	1501
<i>Clostridium scatologenes</i>	2.21	1548
<i>Fermentimonas caenicola</i>	1.33	1562970
<i>Bacteroides salanitronis</i>	1.33	376805
<i>Bordetella bronchiseptica</i>	1.33	518
<i>Clostridium autoethanogenum</i>	0.96	84023
<i>Akkermansia muciniphila</i>	0.89	239935
<i>Bacteroides vulgatus</i>	0.89	821
<i>[Clostridium] stercorarium</i>	0.89	1510
<i>Clostridium acetobutylicum</i>	0.89	1488
<i>Escherichia coli</i>	0.89	562
<i>Eubacterium limosum</i>	0.89	1736
<i>Lachnoclostridium phytofermentans</i>	0.81	66219
<i>Clostridium saccharobutylicum</i>	0.74	169679
<i>Bordetella holmesii</i>	0.70	35814
<i>Achromobacter xylosoxidans</i>	0.44	85698
<i>Bacteroides dorei</i>	0.44	357276
<i>Erysipelothrix rhusiopathiae</i>	0.44	1648
<i>Burkholderia multivorans</i>	0.44	87883
<i>Clostridium carboxidivorans</i>	0.44	217159
<i>Bifidobacterium animalis</i>	0.44	28025
<i>Bordetella parapertussis</i>	0.44	519
<i>Ruminococcus albus</i>	0.44	1264
<i>Butyrivibrio hungatei</i>	0.44	185008
<i>Clostridium cellulovorans</i>	0.44	1493
<i>Odoribacter splanchnicus</i>	0.44	28118
<i>Bordetella bronchialis</i>	0.44	463025
<i>Clostridium tetani</i>	0.36	1513
<i>Clostridium beijerinckii</i>	0.14	1520
<i>Cellulosilyticum lentocellum</i>	0.08	29360

(b) Expected relative abundance for CamiSim Sample 1.

Species	RA (%)	TAXID
<i>Bordetella pertussis</i>	36.65	520
<i>Achromobacter xylosoxidans</i>	14.74	85698
<i>Clostridium botulinum</i>	12.40	1491
<i>Roseburia hominis</i>	8.76	301301
<i>Clostridium perfringens</i>	8.51	1502
<i>Bacteroides fragilis</i>	3.76	817
<i>Clostridium scatologenes</i>	3.59	1548
<i>Bacteroides thetaiotaomicron</i>	2.39	818
<i>Bacteroides dorei</i>	1.82	357276
<i>Clostridium cellulovorans</i>	1.59	1493
<i>Clostridium butyricum</i>	1.26	1492
<i>Ruminiclostridium thermocellum</i>	0.80	1515
<i>Clostridium aceticum</i>	0.75	84022
<i>Parabacteroides distasonis</i>	0.55	823
<i>Butyrivibrio hungatei</i>	0.40	185008
<i>Desulfovibrio africanus</i>	0.40	873
<i>Clostridium pasteurianum</i>	0.40	1501
<i>[Clostridium] stercorarium</i>	0.40	1510
<i>Clostridium saccharobutylicum</i>	0.33	169679
<i>Clostridium sporogenes</i>	0.25	1509
<i>Parabacteroides sp. YL27</i>	0.25	1796646

(c) Expected relative abundance for CamiSim Sample 2.

Pool	Species	Copies ($\times 10^6$)	Volume to Add (uL)	EG (%)	Mix-A (%)	Mix-B (%)	Mix-C (%)	Mix-D (%)	TAXID
Pool A	<i>Escherichia coli</i>	884	100	8.80	30.40	0.04	0.00	2.76	83334
	<i>Staphylococcus aureus</i>	884	53.6	8.80	30.40	0.04	0.00	2.76	1280
	<i>Neisseria meningitidis</i>	884	40.8	8.80	30.40	0.04	0.00	2.76	487
Pool B	<i>Salmonella enterica</i>	768	79	7.60	2.60	30.10	0.03	0.00	28901
	<i>Acinetobacter baumannii</i>	768	63.9	7.60	2.60	30.10	0.03	0.00	470
	<i>Klebsiella pneumoniae</i>	768	100	7.60	2.60	30.10	0.03	0.00	573
Pool C	<i>Streptococcus pyogenes</i>	728	32.3	7.20	0.00	2.90	29.10	0.02	1314
	<i>Achromobacter xylosoxidans</i>	728	100	7.20	0.00	2.90	29.10	0.02	85698
	<i>Shigella sonnei</i>	728	75.3	7.20	0.00	2.90	29.10	0.02	624
Pool D	<i>Enterococcus faecalis</i>	970	65.8	9.60	0.03	0.00	3.90	30.00	1351
	<i>Vibrio furnissii</i>	970	100	9.60	0.03	0.00	3.90	30.00	29494
	<i>Listeria monocytogenes</i>	970	55.8	9.60	0.03	0.00	3.90	30.00	1639
Pool E (controls)	<i>Legionella pneumophila</i>	1363	100	0.14	0.47	0.53	0.54	0.43	446
	<i>Aeromonas hydrophila</i>	1363	71.2	0.14	0.47	0.53	0.54	0.43	644

(d) Expected relative abundances for the NIST mixtures. The final expected DNA concentrations for all members of the EG mixture is 40 ng μ L⁻¹. The final expected DNA concentration for all members of Mix A, B, C, and D is 11 ng μ L⁻¹.

Species	RA (%)		TAXID
	HiLo	Mixed	
<i>Akkermansia muciniphila</i>	0.18	6.37	239935
<i>Alistipes finegoldii</i>	1.30	4.54	214856
<i>Anaerostipes hadrus</i>	1.75	6.11	649756
<i>Bacteroides thetaiotaomicron</i>	7.72	2.69	818
<i>Bacteroides uniformis</i>	1.05	3.66	820
<i>Bifidobacterium longum</i>	37.02	12.92	216816
<i>Blautia wexlerae</i>	0.11	3.77	418240
<i>Clostridium butyricum</i>	10.59	3.70	1492
<i>Collinsella aerofaciens</i>	1.99	6.95	74426
<i>Escherichia coli</i>	9.33	3.26	562
<i>Eubacterium hallii</i>	1.48	5.16	39488
<i>Faecalibacterium prausnitzii</i>	0.16	5.49	853
<i>Lactobacillus gasseri</i>	0.26	8.97	1596
<i>Parabacteroides distasonis</i>	10.10	3.52	823
<i>Prevotella copri</i>	13.84	4.83	165179
<i>Prevotella melaninogenica</i>	1.53	5.34	28132
<i>Roseburia hominis</i>	1.35	4.72	301301
<i>Roseburia intestinalis</i>	0.11	3.88	166486
<i>Ruminococcus gauvreauii</i>	0.12	4.13	438033

(e) Expected relative abundances for the Amos HiLo and Mixed mock communities.

Species	RA (%)	TAXID
<i>Bacteroides uniformis</i>	4.7	820
<i>Blautia sp. NBRC 113351</i>	4.5	2877527
<i>Enterocloster clostridioformis</i>	5.3	1531
<i>Parabacteroides distasonis</i>	4.8	823
<i>Bacillus subtilis</i>	5.2	1423
<i>Streptococcus mutans</i>	6.9	1309
<i>Pseudomonas putida</i>	3.9	303
<i>Lactobacillus delbrueckii</i>	3.6	1584
<i>Escherichia coli</i>	5.6	562
<i>Flavonifractor plautii</i>	3.7	292800
<i>Staphylococcus epidermidis</i>	4.8	1282
<i>Cutibacterium acnes</i>	5.0	1747
<i>Bifidobacterium longum</i>	10.4	216816
<i>Anaerostipes caccae</i>	5.3	105841
<i>Ruminococcus gnavus</i>	5.6	33038
<i>Megasphaera massiliensis</i>	4.8	1232428
<i>Megamonas funiformis</i>	3.7	437897
<i>Collinsella aerofaciens</i>	6.2	74426
<i>Akkermansia muciniphila</i>	6.0	239935

(f) Expected relative abundances for Tourlousse.

Table S1. Expected Relative Abundance tables for all communities.

	bioBakery3	bioBakery4	JAMS	WGS2
bioBakery4	0.092231	-	-	-
JAMS	0.040283	0.003906	-	-
WGS2	0.040283	0.004883	0.965820	-
Woltka	0.003255	0.003255	0.003255	0.003906

(a) Pairwise Wilcoxon test for Aitchison Distance between all pipelines.

	bioBakery3	bioBakery4	JAMS	WGS2
bioBakery4	0.267242	-	-	-
JAMS	0.093355	0.499571	-	-
WGS2	0.093355	0.499571	0.499571	-
Woltka	0.858955	0.852109	0.306084	0.093355

(b) Pairwise Wilcoxon test for Sensitivity between all pipelines.

	bioBakery3	bioBakery4	JAMS	WGS2
Biobakery4	0.777995	-	-	-
JAMS	0.457589	0.777995	-	-
WGS2	1.000000	0.294922	0.457589	-
Woltka	0.009766	0.014648	0.104980	0.061849

(c) Pairwise Wilcoxon test for False Positive Relative Abundance between all pipelines.

Table S2. Pairwise Wilcoxon tests for AD, Sens, and FPRA. The FDR (Benjamini-Hochberg procedure) corrected p-values are shown in the table. Bold values indicate FDR-BH < 0.05.

Parameter	Value
amrfind	false
annotations_type	ko
decontaminate	human_mouse
include_ted_files	false
job_desc	—
mag	false
tax_genes	false
tax_scaffold	false
trim_filter	false

Table S3. Table of WGS2 parameters used.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Aeromonas hydrophila</i>	4	0	1	0	0.800	-	1.000	0.800	0.889	0.0000
<i>Enterococcus faecalis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0000
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Klebsiella pneumoniae</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Salmonella enterica</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0000
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Vibrio furnissii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
Harmonic mean	-	-	-	-	0.000	0.000	0.981	0.675	0.881	0.0000
Mean	-	-	-	-	0.703	0.846	0.984	0.778	0.884	0.0000

(a) Confusion matrix for biobakery3 at threshold 0.0.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Aeromonas hydrophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Enterococcus faecalis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0000
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Klebsiella pneumoniae</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Salmonella enterica</i>	3	1	1	0	0.750	0.000	0.750	0.600	0.750	0.0000
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0000
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Vibrio furnissii</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0000
Harmonic mean	-	-	-	-	0.000	0.000	0.957	0.677	0.889	0.0000
Mean	-	-	-	-	0.733	0.769	0.965	0.792	0.895	0.0000

(b) Confusion matrix for biobakery4 at threshold 0.0.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Aeromonas hydrophila</i>	4	0	1	0	0.800	-	1.000	0.800	0.889	0.0000
<i>Enterococcus faecalis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Klebsiella pneumoniae</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0000
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Shigella sonnei</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0000
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
<i>Vibrio furnissii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0000
Harmonic mean	-	-	-	-	0.828	0.000	0.966	0.836	0.891	0.0000
Mean	-	-	-	-	0.842	0.769	0.971	0.842	0.894	0.0000

(c) Confusion matrix for jams at threshold 0.0.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Acinetobacter baumannii</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Aeromonas hydrophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Enterococcus faecalis</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Klebsiella pneumoniae</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Listeria monocytogenes</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Neisseria meningitidis</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Shigella sonnei</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Staphylococcus aureus</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Streptococcus pyogenes</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Vibrio furnissii</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
Harmonic mean	-	-	-	-	1.000	0.000	0.824	0.824	0.903	0.0000
Mean	-	-	-	-	1.000	0.000	0.828	0.828	0.905	0.0000

(d) Confusion matrix for wgsa2 at threshold 0.0.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Acinetobacter baumannii</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Aeromonas hydrophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Enterococcus faecalis</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Klebsiella pneumoniae</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0000
<i>Listeria monocytogenes</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Neisseria meningitidis</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0000
<i>Staphylococcus aureus</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Streptococcus pyogenes</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0000
<i>Vibrio furnissii</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0000
Harmonic mean	-	-	-	-	0.000	0.000	0.828	0.571	0.906	0.0000
Mean	-	-	-	-	0.800	0.154	0.833	0.731	0.907	0.0000

(e) Confusion matrix for woltka at threshold 0.0.

Table S4. Confusion matrices for bioBakery3, bioBakery4, JAMS, WGS2, and Woltka at 0% filtering threshold. tp = True Positive, fp = False Positive, fn = False Negative, TN = True Negative, sens = Sensitivity, spec = specificity, prec = Precision, acc = Accuracy, f1 = F1 Score

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Aeromonas hydrophila</i>	4	0	1	0	0.800	-	1.000	0.800	0.889	0.0001
<i>Enterococcus faecalis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Klebsiella pneumoniae</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Salmonella enterica</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0001
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Vibrio furnissii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
Harmonic mean	-	-	-	-	0.000	0.000	0.981	0.667	0.872	0.0001
Mean	-	-	-	-	0.687	0.846	0.984	0.764	0.873	0.0001

(a) Confusion matrix for biobakery3 at threshold 0.0001.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Aeromonas hydrophila</i>	4	0	1	0	0.800	-	1.000	0.800	0.889	0.0001
<i>Enterococcus faecalis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Klebsiella pneumoniae</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Salmonella enterica</i>	3	1	1	0	0.750	0.000	0.750	0.600	0.750	0.0001
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0001
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Vibrio furnissii</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
Harmonic mean	-	-	-	-	0.000	0.000	0.957	0.669	0.881	0.0001
Mean	-	-	-	-	0.720	0.769	0.965	0.778	0.886	0.0001

(b) Confusion matrix for biobakery4 at threshold 0.0001.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Acinetobacter baumannii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Aeromonas hydrophila</i>	0	0	5	0	0.000	-	-	0.000	-	0.0001
<i>Enterococcus faecalis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Klebsiella pneumoniae</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Legionella pneumophila</i>	4	0	1	0	0.800	-	1.000	0.800	0.889	0.0001
<i>Listeria monocytogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Neisseria meningitidis</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Shigella sonnei</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Vibrio furnissii</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
Harmonic mean	-	-	-	-	0.000	0.000	0.963	0.000	0.864	0.0001
Mean	-	-	-	-	0.687	0.769	0.969	0.693	0.864	0.0001

(c) Confusion matrix for jams at threshold 0.0001.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Acinetobacter baumannii</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Aeromonas hydrophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Enterococcus faecalis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Klebsiella pneumoniae</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Listeria monocytogenes</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Neisseria meningitidis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0001
<i>Staphylococcus aureus</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Streptococcus pyogenes</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Vibrio furnissii</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
Harmonic mean	-	-	-	-	0.000	0.000	0.963	0.747	0.969	0.0001
Mean	-	-	-	-	0.850	0.769	0.969	0.890	0.972	0.0001

(d) Confusion matrix for wgsa2 at threshold 0.0001.

Species	tp	fp	fn	tn	sens	spec	prec	acc	f1	Threshold
<i>Achromobacter xylosoxidans</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Acinetobacter baumannii</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Aeromonas hydrophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Enterococcus faecalis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Escherichia coli</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Klebsiella pneumoniae</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Legionella pneumophila</i>	5	0	0	0	1.000	-	1.000	1.000	1.000	0.0001
<i>Listeria monocytogenes</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Neisseria meningitidis</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Salmonella enterica</i>	4	1	0	0	1.000	0.000	0.800	0.800	0.889	0.0001
<i>Shigella sonnei</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0001
<i>Staphylococcus aureus</i>	4	0	0	1	1.000	1.000	1.000	1.000	1.000	0.0001
<i>Streptococcus pyogenes</i>	3	0	1	1	0.750	1.000	1.000	0.800	0.857	0.0001
<i>Vibrio furnissii</i>	0	0	4	1	0.000	1.000	-	0.200	-	0.0001
Harmonic mean	-	-	-	-	0.000	0.000	0.941	0.609	0.957	0.0001
Mean	-	-	-	-	0.783	0.692	0.949	0.814	0.960	0.0001

(e) Confusion matrix for woltka at threshold 0.0001.

Table S5. Confusion matrices for bioBakery3, bioBakery4, JAMS, WGS2, and Woltka at 0.1% filtering threshold. tp = True Positive, fp = False Positive, fn = False Negative, TN = True Negative, sens = Sensitivity, spec = specificity, prec = Precision, acc = Accuracy, f1 = F1 Score

	bioBakery3	bioBakery4	JAMS	WGS2	Woltka
Time per CPU (HH:MM:SS)	00:07:23	00:01:09	00:01:33	00:01:43	00:26:45
Relative Time Per CPU (%)	534.4	-	33.0	47.8	2194.5

Table S6. Relative Time per CPU for all NIST samples. If multiple samples were run in parallel, the average was taken. The Woltka time is the sum of both the alignment and classification steps for each sample.

Number False Positive												
	Sample Type	n	bioBakery3		bioBakery4		JAMS		WGS2		Woltka	
			Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
One-to-One	BMock12	1	3		2		31		72		98	
	CamiSim S1	1	3		3		13		21		55	
	CamiSim S2	1	1		1		4		24		75	
	NIST EG	1	5		0		11		14		226	
	NIST MIX-A	1	4		0		3		14		128	
	NIST MIX-B	1	5		1		4		17		180	
	NIST MIX-C	1	3		1		7		16		136	
	NIST MIX-D	1	1		1		2		6		120	
Replicates	Amos HiLo	5	0.20	0.45	0.00	0.00	8.20	0.84	21.80	1.64	117.20	1.92
	Amos Mixed	5	1.00	0.00	0.00	0.00	27.20	1.30	46.20	0.84	212.40	3.91
	Tourlousse	6	7.83	0.41	1.00	0.00	14.17	2.14	35.17	0.41	239.00	3.29
Average			3.09	2.28	0.91	0.94	11.32	9.71	26.11	18.77	144.24	61.75

Table S7. Table of the Number of False Positive species. A perfect score is 0.

Unclassified													
	Sample Type	n	Biobakery3		Biobakery4		JAMS		WGS2		Woltka		Average
			Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev	
<i>One-to-One</i>	BMock12	1	-	-	-	-	1.32	0.00	28.53	0.00	-	-	5.97
	CamiSim S1	1	-	-	-	-	2.25	0.00	0.88	0.00	-	-	0.63
	CamiSim S2	1	-	-	-	-	0.85	0.00	0.08	0.00	-	-	0.18
	NIST EG	1	-	-	-	-	15.70	0.00	0.59	0.00	-	-	3.26
	NIST MIX-A	1	-	-	-	-	3.95	0.00	0.24	0.00	-	-	0.84
	NIST MIX-B	1	-	-	-	-	4.85	0.00	0.25	0.00	-	-	1.02
	NIST MIX-C	1	-	-	-	-	6.42	0.00	0.22	0.00	-	-	1.33
	NIST MIX-D	1	-	-	-	-	2.86	0.00	0.20	0.00	-	-	0.61
<i>Replicates</i>	Amos HiLo	5	-	-	-	-	3.08	0.08	3.34	0.11	-	-	1.28
	Amos Mixed	5	-	-	-	-	7.63	1.55	9.59	0.07	-	-	3.44
	Tourlousse	6	-	-	-	-	3.23	0.30	12.37	0.13	-	-	3.12
Average			0.00	0.00	0.00	0.00	4.74	4.17	5.12	8.85	0.00	0.00	0.00

Table S8. Table of Unclassified metrics. The values shown are percentages. A perfect score is 0%. Neither bioBakery nor Woltka give the number of unclassified by default.

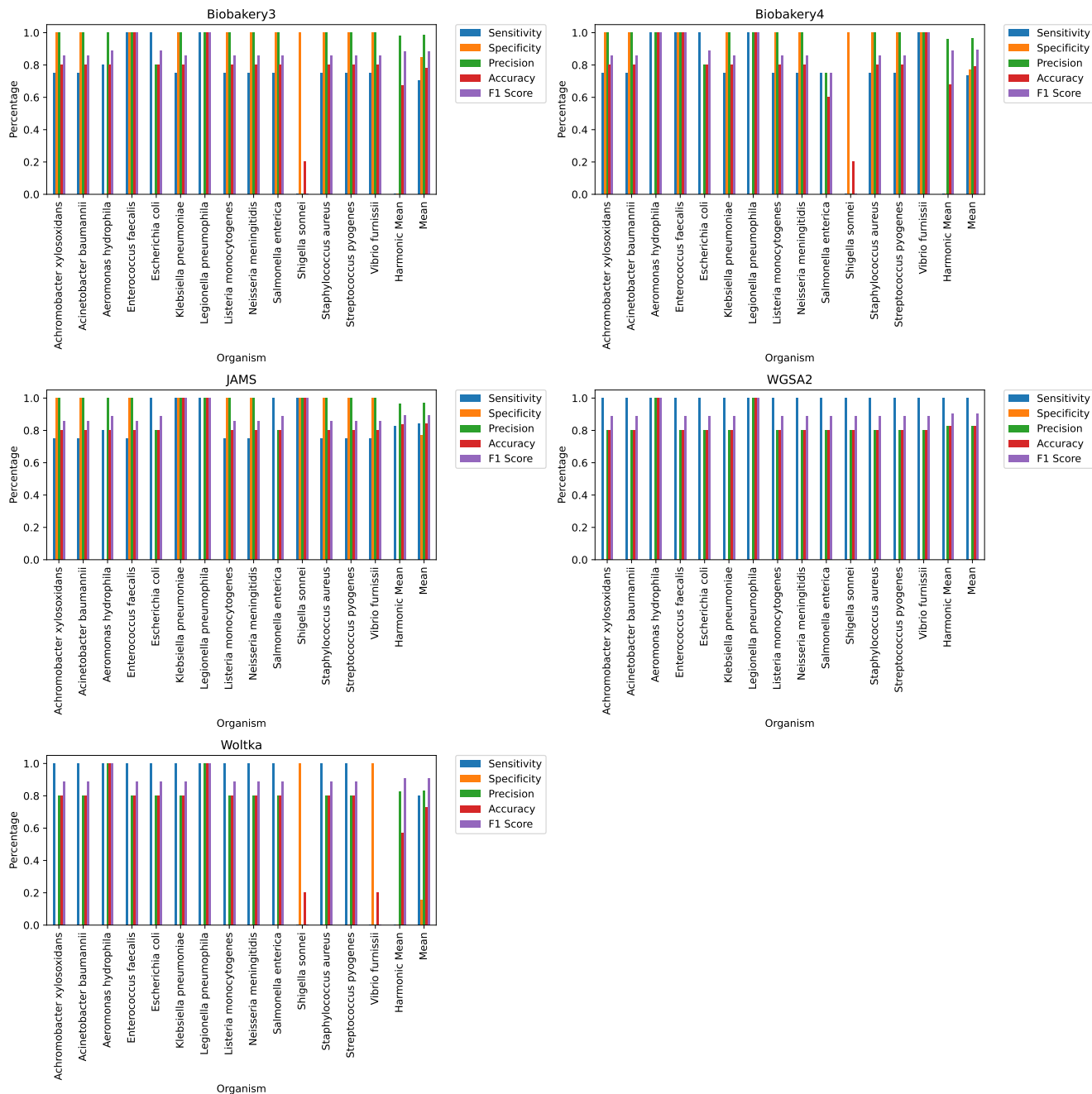


Figure S1. Bar Plot graphical representation of confusion matrices shown in Supplemental Table S4.

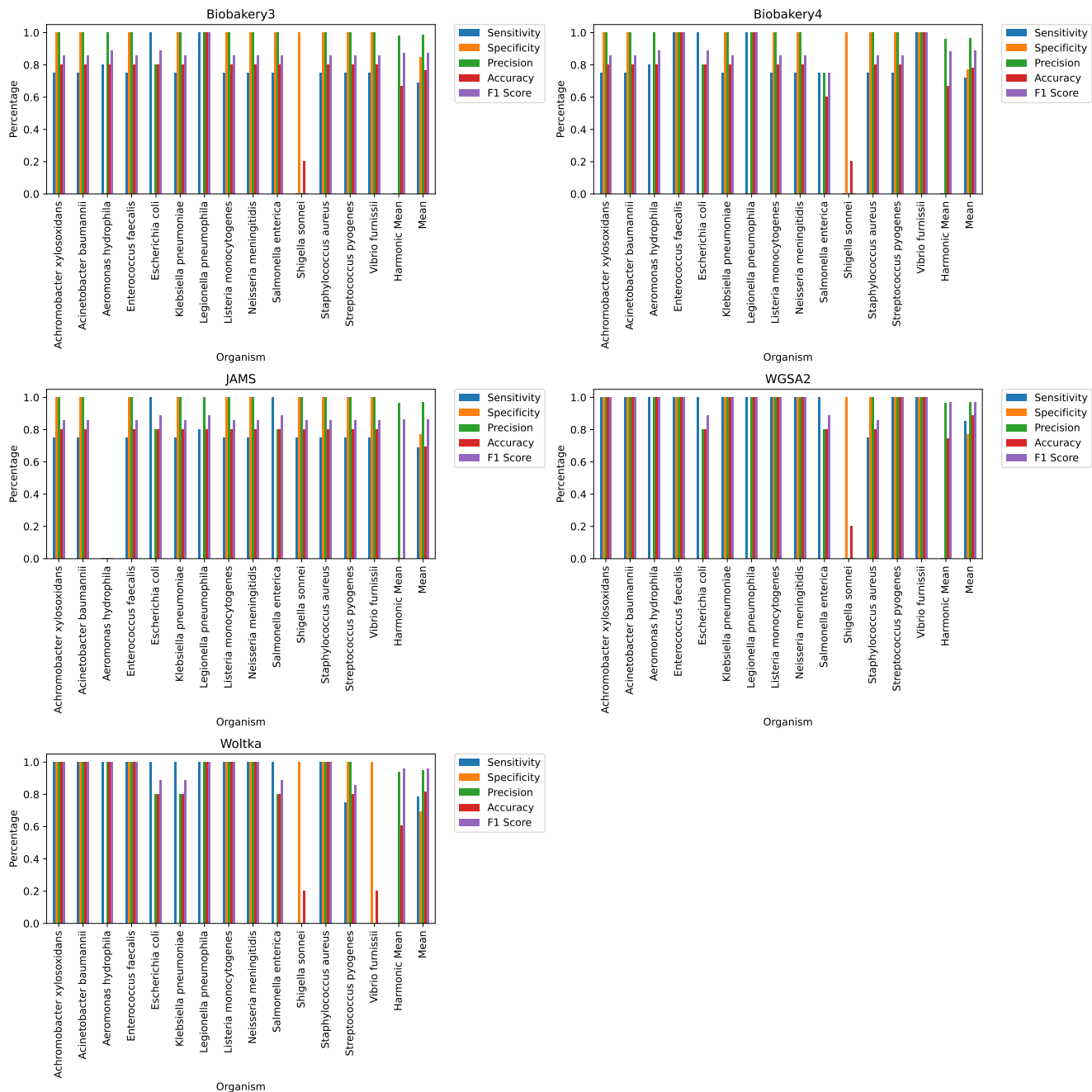


Figure S2. Bar Plot graphical representation of confusion matrices shown in Supplemental Table S5.