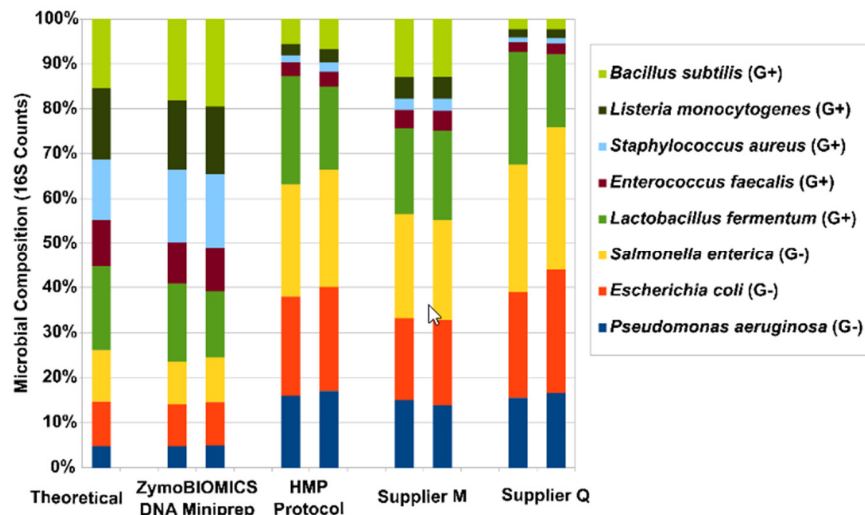


Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures



Supplementary Figure S1. Zymobiomics theoretical spike-in composition based on 16S rRNA amplicon sequencing (1).

1.2 Supplementary Tables

Species	Theoretical Composition (%)					Gram Stain
	Genomic DNA	16S Only	16S & 18S	Genome Copy	Cell Number	
<i>Pseudomonas aeruginosa</i>	12	4.2	3.6	6.1	6.1	-
<i>Escherichia coli</i>	12	10.1	8.9	8.5	8.5	-
<i>Salmonella enterica</i>	12	10.4	9.1	8.7	8.8	-
<i>Lactobacillus fermentum</i>	12	18.4	16.1	21.6	21.9	+
<i>Enterococcus faecalis</i>	12	9.9	8.7	14.6	14.6	+
<i>Staphylococcus aureus</i>	12	15.5	13.6	15.2	15.3	+
<i>Listeria monocytogenes</i>	12	14.1	12.4	13.9	13.9	+
<i>Bacillus subtilis</i>	12	17.4	15.3	10.3	10.3	+
<i>Saccharomyces cerevisiae</i>	2	NA	9.3	0.57	0.29	Yeast
<i>Cryptococcus neoformans</i>	2	NA	3.3	0.37	0.18	Yeast

Supplementary Table S1. ZymoBIOMICS Microbial Community Standard (Zymo Research, Cat no. D6300) consist of 8 bacterial strains (3 Gram-negative and 5 Gram-positive) and 2 tough-to-lyse yeasts at a concentration of 1.4×10^{10} cells/mL (1).

Platform	Dilution Factor	No. of cells per mL of plasma	Categories	% of Reads (HostEL treated)	% of Reads (No treatment)	Fold Enrichment of Microbial reads
iSeq 100	1:100	~1.4 million	Homo sapiens	86.94%	93.18%	-
			Non Human	0.41%	0.23%	
			Zymo Spike-ins	12.65%	6.61%	1.915
	1:250	~560 000	Homo sapiens	90.70%	96.05%	-
			Non Human	0.37%	0.15%	
			Zymo Spike-ins	8.94%	3.80%	2.355
	1:1000	~140 000	Homo sapiens	96.36%	98.99%	-
			Non Human	0.24%	0.15%	
			Zymo Spike-ins	3.40%	0.87%	3.932
	1:2000	~70 000	Homo sapiens	98.46%	99.53%	-
			Non Human	0.21%	0.14%	
			Zymo Spike-ins	1.32%	0.33%	3.952
	No Spike	N.A.	Homo sapiens	99.87%	99.76%	-
			Non Human	0.08%	0.15%	
			Zymo Spike-ins	0.04%	0.09%	0.407
MiniSeq	1:100	~1.4 million	Homo sapiens	83.87%	91.79%	-
			Non Human	0.46%	0.28%	
			Zymo Spike-ins	15.64%	7.91%	1.977
	1:250	~560 000	Homo sapiens	87.76%	95.30%	-
			Non Human	0.46%	0.16%	
			Zymo Spike-ins	11.75%	4.53%	2.594
	1:1000	~140 000	Homo sapiens	93.33%	98.21%	-
			Non Human	2.42%	0.65%	
			Zymo Spike-ins	4.24%	1.13%	3.752
	1:2000	~70 000	Homo sapiens	97.50%	98.19%	-
			Non Human	0.44%	0.51%	
			Zymo Spike-ins	2.05%	1.31%	1.565
	No Spike	N.A.	Homo sapiens	99.50%	98.76%	-
			Non Human	0.15%	1.14%	
			Zymo Spike-ins	0.34%	0.10%	3.4

Supplementary Table S2. Detailed breakdown of percentage of reads of HostEL treated vs no treatment enrichment on iSeq 100 and MiniSeq sequencing platforms. HostEL can enrich the percentage of reads by ~2 fold in plasma with high microbial concentrations (1.4 million cells/mL) and ~4 fold with low microbial concentration (140,000 cells/mL and 70,000 cells/mL) on iSeq 100 sequencing platform while enriching at least 1.5 fold on MiniSeq sequencing platform.

Pathogen	Primer	Primer Sequence	Reference
BK virus	F	5'-AGT GTT GAG AAT CTG CTG TTG CTT-3'	(2)
	R	5'-GGG ATG AAG ATT TAT TTT GCC ATG AAG AT-3'	
Dengue Virus	F	5'-GCA TAT TGA CGC TGG GAR AGA C-3'	(3)
	R (DENV1-3)	5'-TTC TGT GCC TGG AAT GAT GCT G-3'	
	R (DENV4)	5'-YTC TGT GCC TGG ATW GAT GTT G-3'	
Hepatitis B virus	F	5'- TTC CGG AAA CTA CTG TTG TTA GAC-3'	(4)
	R	5'- ATT GAG ATT CCC GAG ATT GAG A-3'	
Hepatitis C virus	F	5'-GTC TGC GGA ATC GGT GAG TAC A-3'	(5)
	R	5'-CTA TCA GGC AGT ACC ACA AGG-3'	
Hepatitis E virus	F	5'-AAT AAA TCA TAA GGT GGT TTC TGG GGT GAC-3'	(6)
	R	5'-AAT AAA TCA TAA GGG GTT GGT TGG ATG AA-3'	
Varicella-Zoster Virus	F	5'-CAA AGC GGG TCC ATC CCT-3'	(7)
	R	5'-ACC TGG CCT TTG CCG G-3'	

Supplementary Table S3. List of targeted primers used in qPCR validation of clinical samples.

Samples	qPCR Ct Value	Frac Reads	Raw Reads Counts	Platform	Clinical Diagnosis
B047	43.8	0.00E+00	0	iSeq 100	Hepatitis B virus
B046	UD	0.00E+00	0		
B050	UD	0.00E+00	0		
B048	38.6	8.00E-05	2		
B049	29.5	8.05E-03	118		
B017	24.4	2.11E-02	495		
B016	17.9	8.64E-02	4238		
B045	22.1	3.32E-01	7427		
B047	43.8	0.00E+00	0	MiniSeq	
B046	UD	0.00E+00	0		
B050	UD	0.00E+00	0		
B048	38.6	2.00E-05	2		
B049	29.5	7.80E-03	318		
B017	24.4	0.02203	1226		
B016	17.9	0.09309	11394		
B045	22.1	3.77E-01	23336		

Supplementary Table S4. qPCR and iSeq 100/MiniSeq sequencing results of Hepatitis B plasma samples

2 References

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