SUPPLEMENTARY MATERIALS

Supplemental Methods

Supplemental Method 1: CNASafe cartridge assembly

The fluidic channels (which are featured in both sides of the fluidic side) were closed by welding of a thin COC layer to each side of the cartridge. Furthermore, the fluidic side is fitted with needle-like structures (1.5 mm OD, 0.8 mm ID, 1.8 mm height) which allow the piercing of the front face foil of the reagent side, thus opening the fluidic path within the cartridge. Placed around each of these piercing structures is a soft ring which guarantees fluidic sealing while the two sides are pressed together. These O-rings are toroids derived by punching a 1mm 30˚ silicon layer (Silex ltd) with a 3mm and a 1.5 mm biopsy punch.

The reagent side of the CNASafe cartridge was prepared by drilling two 1.6mm filling holes into each reagent chamber before the front face was sealed with LINC 1298 Heat Seal Foil (Label Innovation). Sealing was carried out by a third party until 12/05/2021, after which sealing was carried out in-house. Reagent chambers were filled with proprietary extraction reagents and sealed with QuickSeal Foil PCR Ultra™ Self Adhesive Sealing Film (IST Scientific) prior to experiments. The optimisation of elution buffer volume reported in the Results section was performed prior to the sealing being carried out in-house. All other results were carried out after sealing was done in-house.

The custom alignment frame feature was designed with the multiple aims to 1) aligning the two sides of the cartridge 2) avoiding unwanted piercing during transportation and 3) keeping the parts together after the CNA extraction to avoid potential spillage of liquid in the case of incorrect emptying of the cartridge. The two cartridge sides are initially separated by the alignment feature, which is meant to allow safe transport of the cartridge maintaining the two parts connected but separated.

The membrane (Mini Spin column (Qiagen)) was prepared by piercing a small hole in the lid of the column and positioning rigid tubing (ID 0.8 mm, length 75 mm) just above the membrane. The tubing was secured into place and the lid of the column sealed using epoxy glue (Loctite 3430, Henkel). All prepared columns were pressure-tested prior to use for quality control. Only columns with a determined dry baseline pressure drop of 7.4 +/- 1.1 kPa were used. The external membrane along with tubing for the connection to the sample tube, was attached to the fluidic side before the two sides of the cartridge (reagent and fluidic) were loaded onto a custom automation platform. Here the two sides are brought together with the aid of an alignment frame, resulting in the piercing of the front face foil (of the reagent cartridge) by the needle-like projections on the fluidic side.

Supplemental Method 2: cfDNA extractions on CNASafe device

Extractions were performed using the CNASafe cartridge. The extraction reagents used in the cartridge were adapted from the QIAamp Circulating Nucleic Acid Kit (Qiagen) in a proprietary fashion. Depending on the experimental condition (cartridge performance or workflow validation), and as individually detailed, cfDNA was extracted from 300 µL - 4 mL plasma in a total starting volume of 4 mL (volume made up to 4 mL with DPBS) and eluted in 65 -110µL an elution buffer. The mixing time of plasma with extraction reagents (PK, Lysis and Binding buffers) varied from 3 - 12 min for cartridge performance experiments and a mixing time of 3 min used for workflow validation experiments. The total extraction takes between 40 to 45 minutes for a 3 min mixing time. Before starting the extraction protocol, the two sides of the cartridges are pressed together by the

clamping system in the automation platform and a sample tube, containing 1 to 4 mL of human plasma, is connected to the cartridge fluidic side. The protocol is initiated from a laptop.

Investigation on elution volumes. To assess this, cfDNA was extracted from 1 mL human pooled plasma samples (mixing time 3 min), in an initial volume of 4 mL (1mL plasma + 3mL DPBS). Reagent chambers contained reagent volumes appropriate to a 4 mL starting sample. Figure 3.A represents the cartridge eluate volume extracted from the cartridge and the total cfDNA normalised to manual extraction, with the volume of elution buffer added to the cartridge: 65, 100 and 110 µL.

Investigation on mixing time and silica membrane lot number. The optimum sample mixing time was investigated, considering cfDNA recovery from 1 mL plasma sample in a total starting volume of 4mL (1mL Plasma + 3mL DPBS). Reagent chambers contained reagent volumes appropriate to a 4 mL starting sample. Secondly, for each sample, we compared the cfDNA extractions using the CNASafe cartridge and its silica membrane, with the manual extraction method using a membrane from the same lot and the same biological samples. Results are represented in Figure 3.B. The QIAamp Mini columns used here were from 3 different lot numbers (#a, #b and #c).

Investigation on plasma and DPBS sample volume. We considered 1, 2, 3 and 4 mL of plasma (mixing time 6 min) with 1- 4 mL of DPBS. For all cartridge extractions performed, reagent chambers contained reagent volumes appropriate to a 4 mL starting sample. The total cfDNA amount obtained from each cartridge extraction was normalised against the manual extraction result for the same plasma volume, as illustrated in Figure 3.C.

Investigation on storage duration. To determine whether the CNASafe cartridge and its reagents could withstand long-term storage, cartridges were sealed in-house and stored with the sealing foil face up, at room temperature or 4°C, for 9 months. At months 1-9, cartridges from each storage temperature were used to extract cfDNA from 1 mL of plasma in a total starting volume of 4mL (1mL Plasma + 3mL DPBS).

Supplemental Method 3: qPCR quality controls

qPCR analysis was performed using StepOne™ Software v2.1. Outliers were identified as having a Cq valve of >0.3 between technical replicates. Cq values of NTC were above 30 with average Cq values for standards ranging from 10.17 (10ng/µL) to 20.92 (0.01ng/ul), resulting in an average r^2 of 0.997 across runs. A melting curve was performed in all experiments as a control measure for non-specific amplification. A standard curve was created using Human Genomic DNA (Bioline). Absolute amounts of cfDNA in each sample (cartridge and benchmark eluates) were obtained from the standard curve.

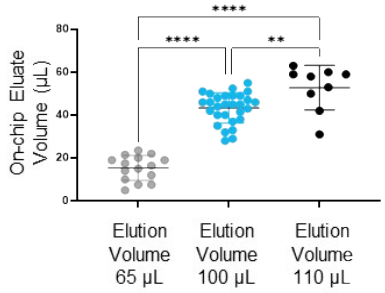
Supplemental Method 4: Compatibility of CNASafe technology to nanopore sequencing workflow.

To verify the compatibility of CNASafe cfDNA extraction with downstream nanopore sequencing, we created mock samples by mixing 1.5 ng of fragmented microbial community mix to 1 mL of stored human plasma (Cambridge Bioscience). The community mix containing genomes from 8 microbes (7 bacteria and 1 fungus) (ZymoBIOMICS HMW DNA Standard (D6322), Zymo Research) was fragmented by dsFragmentase (M0348, NEB) treatment according to the manufacturer's protocol for fragments under 200 bp: 35 min incubation at 37°C followed by clean up using SPRIselect magnetic beads (Beckman Coulter). cfDNA was extracted from the same samples both with the manual

reference method and with the automated CNASafe protocol using a 3 min mixing time. The eluates were then sequenced as described in the method section. Three performance indicators (classified reads, percentage of unclassified reads and Phred score) were quantified to compare benchmark (B) and cartridge (C) extractions (Supplemental Fig S3.A-C). All histograms showed no significant differences between the two extraction protocols. For all samples analysed, the percentage abundance for the eight microbes of the community mix show similar patterns between the bench and the cartridge extractions, while controls (plasma without spiked microbial mix) showed no hit (Supplemental Figure S3.D). These results confirm the compatibility of the CNASafe extraction with downstream sequencing but also indicate the capability of our integrated assay for further deployment of sepsis detection in the field. In addition, the negative controls show that the on-chip cfDNA extraction does not introduce contaminants.

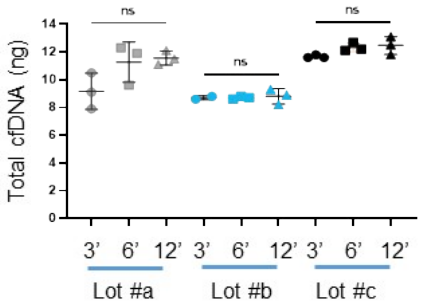
Supplemental Figures

Supplemental Figure 1



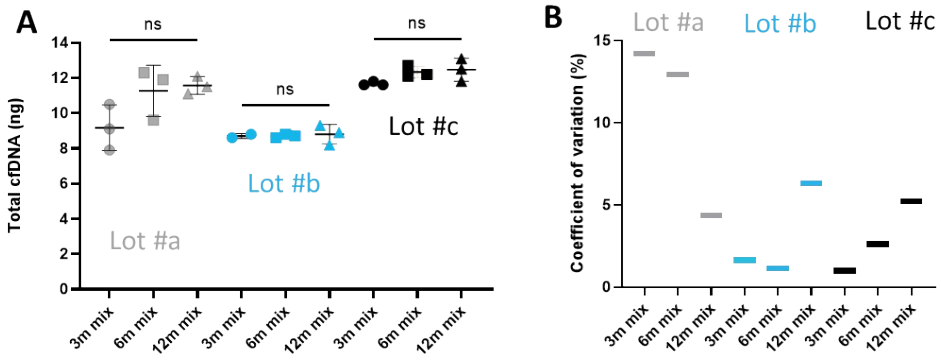
Supplementary Figure 1: Impact of on-chip elution volume on eluate volume.

Supplemental Figure 2

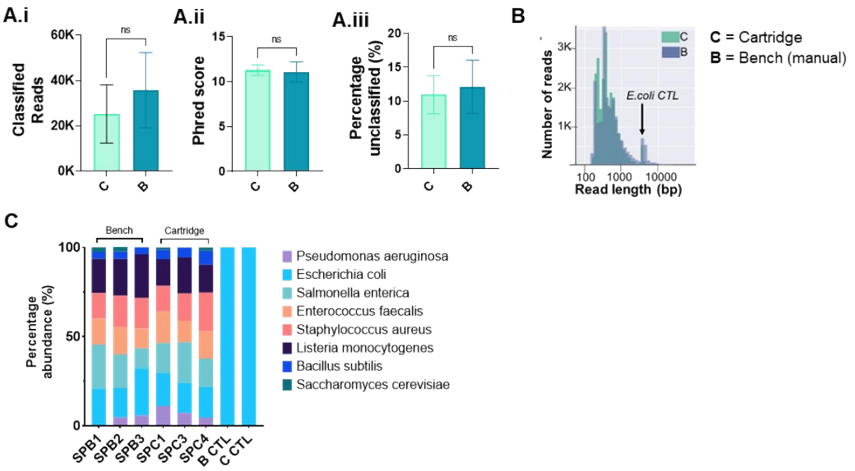


Supplementary Figure 2: Impact of lot number and mixing time on amount of cfDNA (ng).

Supplemental Figure 3



Supplementary Figure 3: (A) Total amount cfDNA (ng) for different Lot number and mixing time and (B) Corresponding coefficient of variations



Supplemental Figure 4

Figure S3: Characterisation of sequencing workflow on Cartridge (C and Benchmark eluates). ***(A)*** *Comparison of Classified reads (A.i), Phred score (A.ii) and Percentage of unclassified reads (A.iii) between cartridge (C) or bench (B) extractions (N=3).* ***(B)*** *Comparison of number of reads vs read lengths for Cartridge and Bench eluates* ***(C)*** *Relative abundance of microbial mix in bench (SPB1-3) and cartridge (SPC1, 3-4) extracted eluates for each of the 8 microbe genomic DNA included in the community mix. Plasma only controls on Bench and Cartridge (B CTL and C CTL) show the presence of an E.Coli internal control.*

Supplementary Tables

Supplementary Table S1

Sample ID	Starting plasma volume (ul)	Extraction eluate volume (µl)	Base-calling mode / Qscore	Min KNOW Version	Guppy Version	iSEP-SEQ Results (with Pathdet bioinformatic workflow)					Blood culture results	Comparison between blood culture results and iSEP-SEQ
						Total Reads Analysed	Number Non-human Reads	Identified organisms (Species, 3 reads or more)				
								Name	Number of reads	RPM		
CRISP 1	1000	52	HA / 0	21.02.2	4.3.4	479098	26158	none	-	-	'Negative'	Consistent
CRISP 2	1000	54	HA / 0	21.02.2	4.3.4	230872	15907	Human gammaherpesvirus 8	3	13	'Negative'	iSEP-SEQ identification of HHV8
CRISP 4	1000	53	HA / 0	21.02.2	4.3.4	84043	8154	Human gammaherpesvirus 8 Arcobacter cryaerophilus Rhizobacter gummiphilus Cutibacterium acnes	5 5 3 3	59 59 35 35	'Positive', 'gpc seen', 'CoNS'	iSEP-SEQ identfication ofHHV8 and A.cryaerophilus below threshold, but plausible.
CRISP 5	950	56	HA / 0	21.02.2	4.3.4	47082	2061	none	-	-	'Positive' 'gnb seen' 'K. pneumoniae'	iSEP-SEQ false negative due to small number of reads
CRISP 6	1000	62 (spin eluate)	HA / 0	21.02.2	4.3.4	209971	23903	Methylobacterium_sp._XJLW Methylobacterium_phyllosphaerae Methylobacterium_oryzae Human betaherpesvirus 5	366 202 55 3	1743 962 261 14	'Positive' 'gpc seen' 'CoNS'	iSEPSEQ identification of Methylobacterium plausible given the strong signal
CRISP7	300	50	HA / 0	21.02.2	4.3.4	292367	19067	Cutibacterium acnes	4	13.7	'Negative'	Consistent
CRISP 8	1000	48	HA / 0	20.06.17	4.0.11	321387	27838	Streptococcus_pneumoniae Methylobacterium sp. XJLW Methylobacterium phyllosphaerae	124 6 4	385 18 12	'Positive'– 'gnb seen' 'No growth but gpc seen'	iSEP-SEQ identification of S.pneumoniae
CRISP 9	1000	53	HA / 1	21.11.7	5.1.13	222499	15878	Methylobacterium sp. XJLW	4	18	'Negative'	Consistent
CRISP 10	625	56	HA / 1	21.11.7	5.1.13	386144	16863	Methylobacterium sp. XJLW Methylobacterium phyllosphaerae Methylobacterium oryzae CBMB20	33 17 3	85 44 7	'Negative'	Consistent

Supplementary Table S2: Extended sequencing data for clinical patient samples in the CRISP iSEP-SEQ study

Supplementary Table S2

Sample #	Epi2me Data										
	Total Reads (all)	Reads failed Qscore filter (Q7)	Reads Analysed	Reads Classified	Reads Unclassified	% unclassified (unclassified/Analysed)*100	Ave Segence Length	Ave Quality Score	Cumulative Reads Homo sapiens	Identified organisms (Species level, 3 reads or more)	
										Name	No. of Reads
1	957,362	64,471	892,890	880,089	12,801	1.4	506	11.41	880,050	-	-
2	230,872	23	212,297	209,362	2,935	1.4	468	11.44	209,324	Human gammaherpesvirus 8	
4	84,043	7,755	76,286	72,516	3,770	4.9	473	10.93	72,386	Human gammaherpesvirus 8 Arcobacter cryaerophilus Rhizobacter gummiphilus Cutibacterium acnes	6 4 3 3
5	47,082	2,682	44,398	43,088	1,310	3.0	519	10.85	43,073	-	-
6	209,971	14,073	195,898	176,554	19,344	9.9	401	10.66	175,068	Methylobacterium sp. XJLW Methylobacterium phyllosphaerae Methylobacterium oryzae	178 136 118
7	292,367	18,937	273,425	265,775	7,650	2.8	514	11.42	265,679	Cutibacterium acnes Flavobacterium sp. KBS0721 Methylobacterium sp. XJLW Proteus mirabilis	5 3 3 3
8	321,387	32,131	289,255	283,976	5,279	1.8	550	10.61	283,311	Streptococcus pneumoniae Methylobacterium phyllosphaerae Methylobacterium sp. XJLW	517 9 9
9	222,499	19,327	203,172	200,354	2,818	1.4	525	11.2	200,332	Methylobacterium sp. XJLW	3
10	386,144	20,540	365,604	360,640	4,964	1.4	459	12.46	360,512	Methylobacterium phyllosphaerae Methylobacterium sp. XJLW Methylobacterium oryzae	26 22 9

Supplementary Table S2: Statistics for EPI2ME bioinformatic workflow

Supplementary Table S3

Nextflow configuration		Sample Id /Run name	Water_17Nov25_2_S10_6h	Water_17Nov25_2_S5_6h	Water_17Nov25_2_S1_6h	Water_17Nov25_2_G10_6h	Water_17Nov25_2_G5_6h	Water_17Nov25_2_G1_6h
Input Options		Select path to FASTQ files	Water_17Nov25_2	Water_17Nov25_2	Water_17Nov25_2	Water_17Nov25_2	Water_17Nov25_2	Water_17Nov25_2
		Classification Method	Kraken2	Kraken2	Kraken2	Kraken2	Kraken2	Kraken2
		Analyse unclassified reads	✓	✓	✓	✓	✓	✓
Reference Options		Database	PlusPF-8	PlusPF-8	PlusPF-8	PlusPF-8	PlusPF-8	PlusPF-8
		Taxonomic rank	S	S	S	G	G	G
Kraken2 Option		Bracken minimum read threshold	10	5	1	10	5	1
		Bracken Length	100	100	100	100	100	100
		Enable memory mapping	✓	✓	✓	✓	✓	✓
		Confidence score threshold	0	0	0	0	0	0
Report Options		Abundance threshold	1	1	1	1	1	1
Advanced Options		Min read length	0	0	0	0	0	0
		Min read quality	1	1	1	1	1	1
		Max read length	-	-	-	-	-	-
Results	% unclassified reads		98.39	98.39	98.39	98.39	98.39	98.39
	Genus Hits (excl. human)	Genus	-	-	-	-	-	-
		No. Reads						
		RPM	0	0	0	0	0	0
		% of total classified reads	0.000	0.000	0.000	0.000	0.000	0.000
	Species Hits (excl. human)	Species	-	-	-			
		No.Reads	-	-	-			
		RPM	-	-	-			
		% of total classified reads	0.000	0.000	0.000			
	Total Reads (from report)		27,323	27,323	27,323	27,323	27,323	27,323
	Total Unclassified Reads		26,882	26,882	26,882	26,882	26,882	26,882
	Total Classified Reads		441	441	441	441	441	441
	Total human Reads		438	438	438	438	438	438
	Total non-human Reads		3	3	3	3	3	3
Kingdom	Bacteria -Total Reads (from adundance table, Genus level)		-	-	-	-	-	-
	Fungi -Total Reads (from adundance table, Genus level)		-	-	-	-	-	-
	Virus -Total Reads (from adundance table)		-	-	-	-	-	-
	Eukaryota Incertae sedis -Total Reads (from adundance table)		-	-	-	-	-	-

Supplementary Table 3: Epi2me raw data and statistics for blank water sample (negative control). Workflow: wf-metagenomics. Workflow Version: 2.13.0; Mean Read Quality4.7; Mean Read Length:102.8

Supplementary Table S4

Supplementary Table 4: Comparison of cfDNA extraction state of the art.

Paper	Sample Types	Processing Volume	Elution Volume	Application Domain	Automation Level	Duration of the extraction	DNA Quantitative Characterisation	cfDNA Recovery characterisation method	cfDNA Recovery (Caution with interpretration: Not for comparison unless same method used)	Benchmark choice and method	Comparison to benchmark (Caution with interpretration: Not for comparison unless same method used)
Yang et al. 2015 10.1039/C5LC00681C Lab on a Chip	Plasma, Whole blood (patients with severe sepsis and healthy controls)	<10 µL droplet	Not specified	Sepsis, ICU mortality prognosis	Semi-automated	5 min	PicoGreen fluorescence labeling, Electrophoresis	Not available	n/a	Not mentioned	n/a
Kim et al. 2018 10.1039/C8LC00165K Lab on a Chip	Whole blood (>3 mL), Plasma from NSCLC patients	>3 mL whole blood	Not specified	Cancer (non-small cell lung cancer), Cancer therapy monitoring	Fully automated	30 min	qPCR, Droplet digital PCR	Synthetic short DNA (300 base pairs) spiked into	50-75%	Qiagen QIAamp DNA Blood Mini Kit (Q-BM) and QIAamp cfDNA Kit (Q-cfNDA) Synthetic short DNA	Similar performance
Kye et al. 2020 10.1007/s13206-020-4208-1 BioChip Journal	Synthetic cfDNA at a concentration of 50 ng/mL and 250 ng/mL in DEPC-DW with 4,000 ppm PEO (Sigma	1 mL sample volume	50 µL	Cancer diagnostics, Cancer prognosis	Semi-automated	At max flowrate	PCR amplification, Gel electrophoresis	Not available	n/a	Not specified	n/a
Campos et al. 2018 10.1039/C8LC00716K Lab on a Chip	Plasma from healthy donors, NSCLC patients, Colorectal cancer patients	Not clearly stated. Paper states ambition to process > 5mL	Not explicitly stated	Cancer (NSCLC, colorectal cancer), Oncology	Manual / Semi-automated (pre-	At max flowrate	Qubit quantification, Ligase detection reaction (LDR) for KRAS mutations, qPCR	Method 1: Using a DNA ladder consisting of 50-700 bp double-stranded DNA fragments. DNA ladder was spiked into PBS (concentration	Method 1: >90% for 100-700bp; >70% for 50bp Method 2: 92% recovered	Benchmarks: Norgen and Qiagen cfDNA kits. Method: Using a DNA ladder consisting of 50-700 bp	Similar performance
Gwak et al. 2019 10.1063/1.5100009 Biomicrofluidics	cfDNA-spiked PBS at 1 ng/ml, Serum from breast cancer patients and healthy	500 µL serum	50 µL	Cancer (breast cancer), Cancer diagnostics	Semi-automated	19 min	Qubit quantification, Real-time PCR, qPCR	cfDNA-spiked (IL2) PBS at 1 ng/ml characterised	51.80%	Benchmark: MagMAX Cell-Free DNA Isolation Kit (Applied Biosystems) - Should be noted	Similar performance
Lee et al. 2020 10.1038/s41698-019-0107-0 npj Precision Oncology	Plasma; Synthetic plasma with cfDNA reference standards	0.5-1 mL plasma	50-150 µL (adjustable)	Cancer (HER-2 breast cancer with liver metastasis), Cancer monitoring	Fully automated	15 min	ddPCR, NGS, qPCR	DNA extraction efficiency was calculated based on specific DNA recovery by comparing the absolute copy number of DNA detected in qPCR to the theoretically expected value assuming	70-80%	Benchmark: Qiagen QIAamp cfDNA. Method: The comparative extraction efficiency between QIAamp and PIBEX chip was	Similar performance
Schneider et al. 2022 10.1002/pd.6092 Prenatal Diagnosis	cfDNA in AcroMetrix EDTA Plasma Dilution Matrix	1 ml of AcroMetrix EDTA Plasma Dilution Matrix	10-50 µL typical	Prenatal diagnosis (NIPT), Prenatal screening	Semi-automated	20 min	Agilent Bioanalyzer	cfDNA eluate sample yield was performed using	60-70%	Not mentioned	n/a
Hu et al. 2019 10.1039/C9AN00493A Analyst	Whole blood (4 mL)	4 mL whole blood 1.6 mL plasma	Not specified	Not specified (liquid biopsy applications)	Semi-automated	15 min	qPCR, Droplet digital PCR	T790 M mutation (varying degrees of mutation including 5%, 20%, and 50%) spiked in the whole blood in ctDNA isolated from whole blood	30-65%	Benchmark: MagMAX Cell-Free Total Nucleic Acid Kit. Method: T790 M mutation (varying degrees	Similar performance
Jin et al. 2018 10.1002/advs.201800614 Advanced Science	Spiked buffer, 14 samples from prospective CRC patients, and 10 from	500 µL plasma samples	Not specified	Cancer (colorectal cancer), KRAS and BRAF mutation detection	Semi-automated	20 min	qPCR	200 µL of the ALU gene (247 bp amplicon) was mixed with 100 µL of DTBP (100 mg/mL) and then was injected into the platform. PCR (only	1 Ct cycle difference between input and ch	Benchmark: Qiagen QIAamp cfDNA Kit . Method: Characterised on plasma	Information in the paper is conflicting and no statistical analysis
This paper	Plasma from healthy donors (400 samples) and hospital patients with suspected sepsis (9 patients)	1-4 mL plasma	50 µL	Sepsis, Pathogen identification	Fully automate	40 min	qPCR, mNGS	Identical healthy human plasma samples processed in same volume on the benchmark manual extraction and microfluidic device. qPCR on LINE sequence. Recovery expressed as percentage relative to benchmark amount	Relative recovery to benchmark is 100% (averaged over 333 samples)	Benchmark: Qiagen QIAamp cfDNA kit. Method: Characterised with quantitative PCR on LINE sequence on matched 1-4mL plasma samples (biological replicates N=458, considered for	Similar performance