Supporting Information

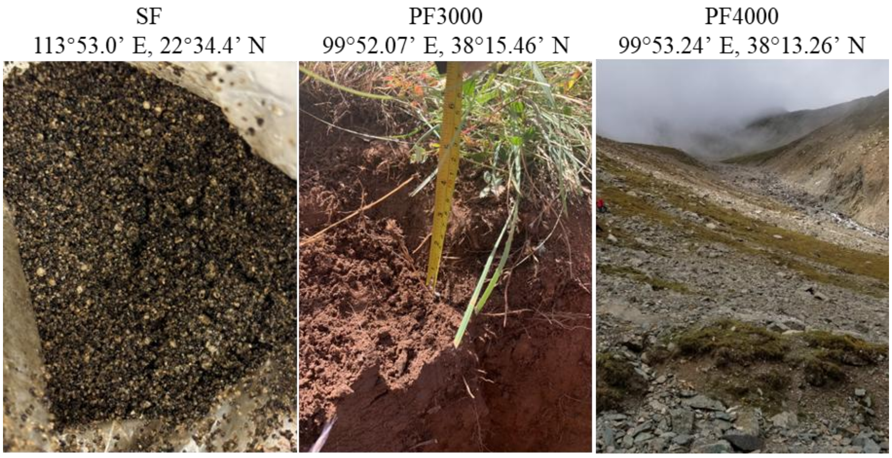


Fig. S1 Sampling point location and picture.

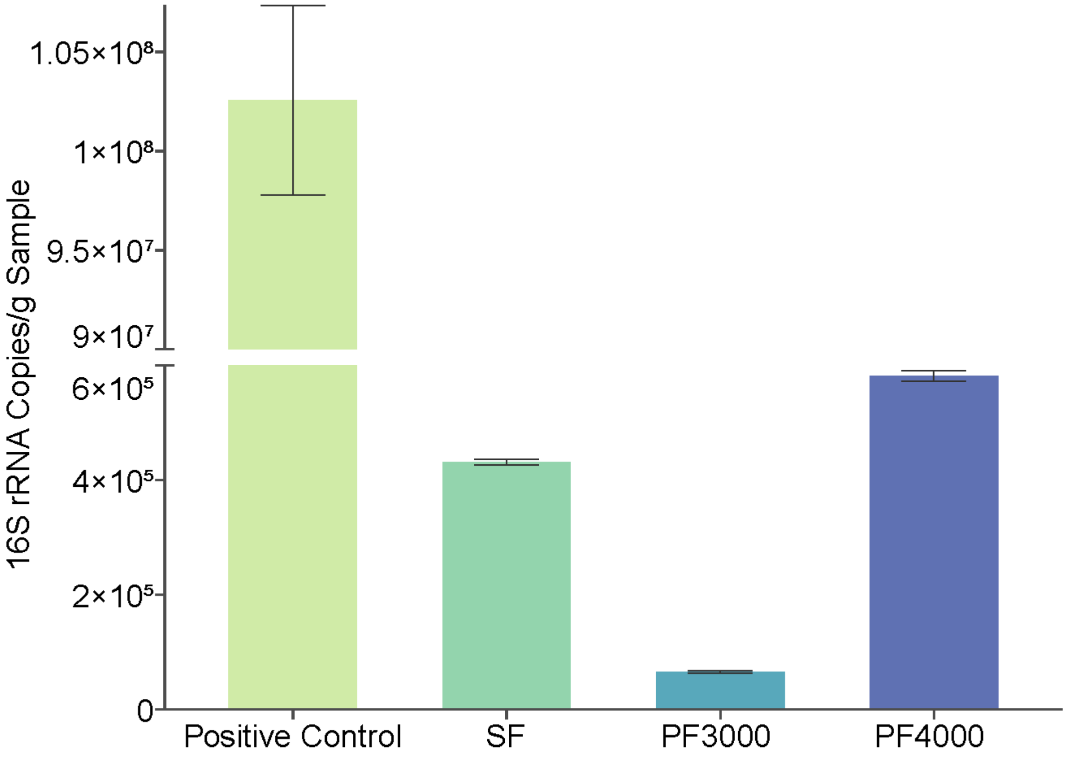


Fig. S2 The absolute quantitative results of qPCR for the samples, with the positive control using activated sludge sample.

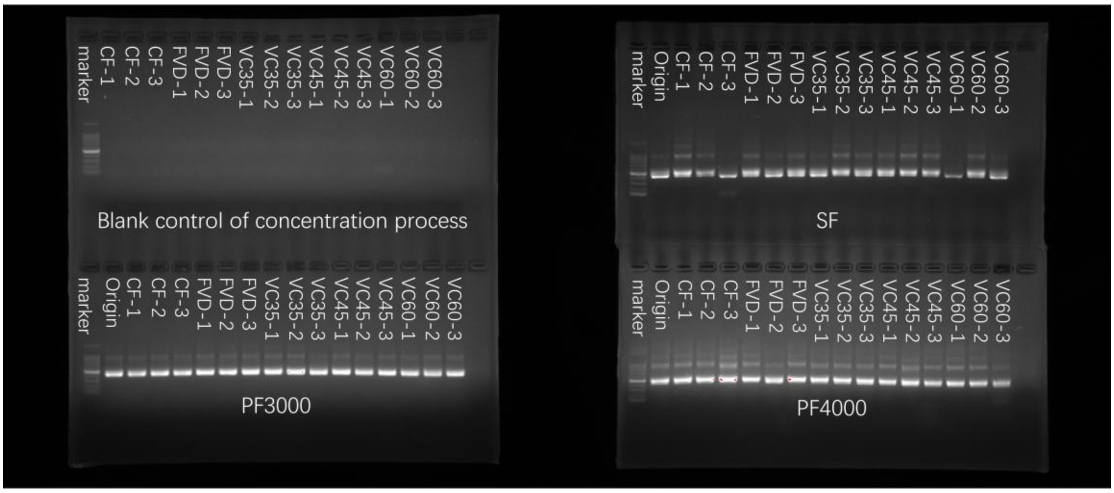


Fig. S3 PCR gel electrophoresis of enriched blank and sample.

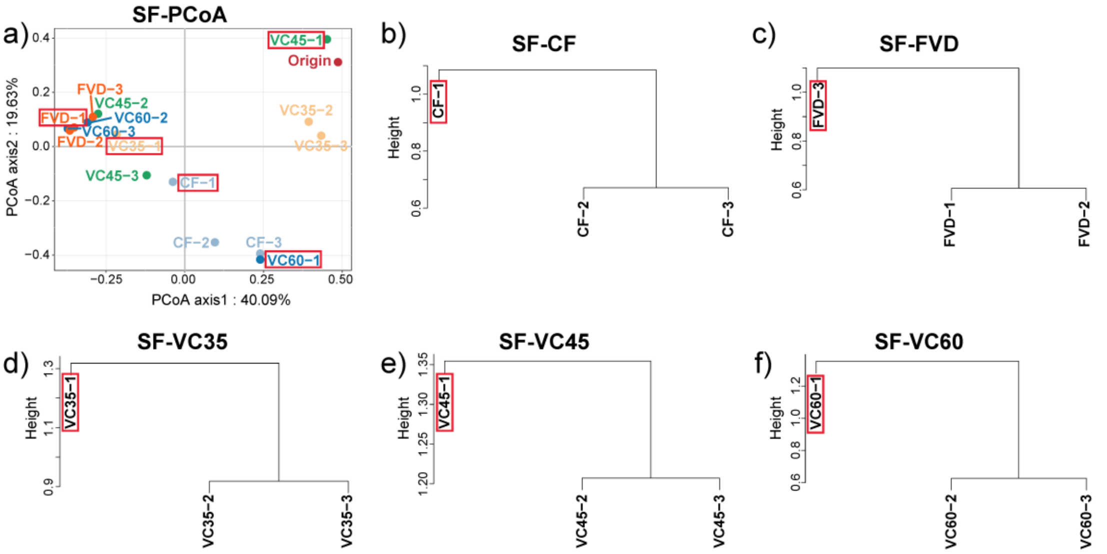


Fig. S4 Replicate screening of SF amplicon samples. (a) PCoA of the samples before screening. (b) – (f) show the clustering relationships of the replicate samples for the CF, FVD, VC35, VC45, and VC60 methods, respectively. The red box line marks things outliner.

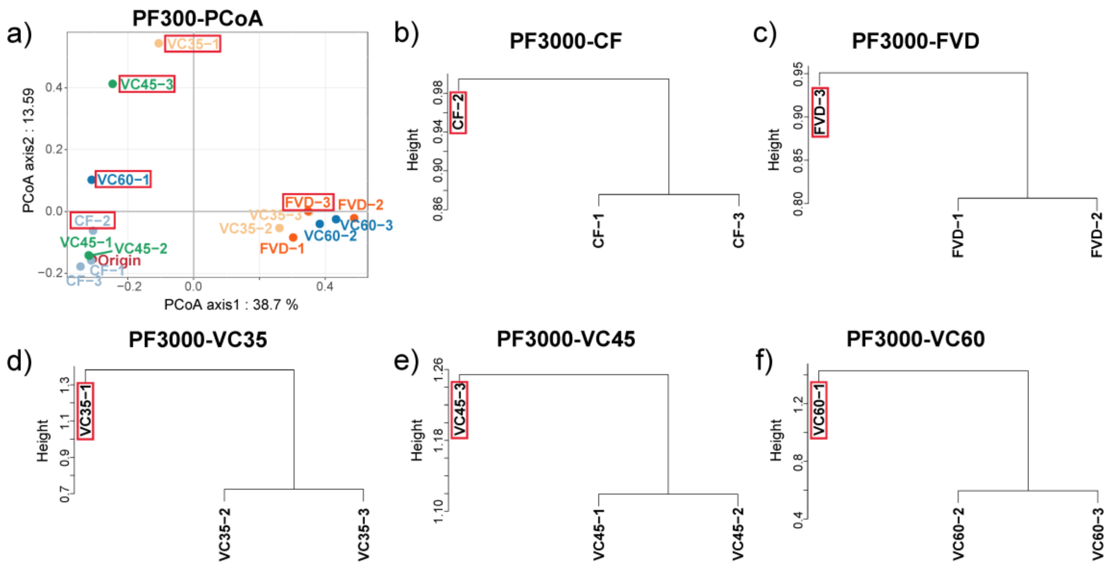


Fig. S5 Replicate screening of PF3000 amplicon samples. (a) PCoA of the samples before screening. (b) – (f) show the clustering relationships of the replicate samples for the CF, FVD, VC35, VC45, and VC60 methods, respectively. The red box line marks things outliner.

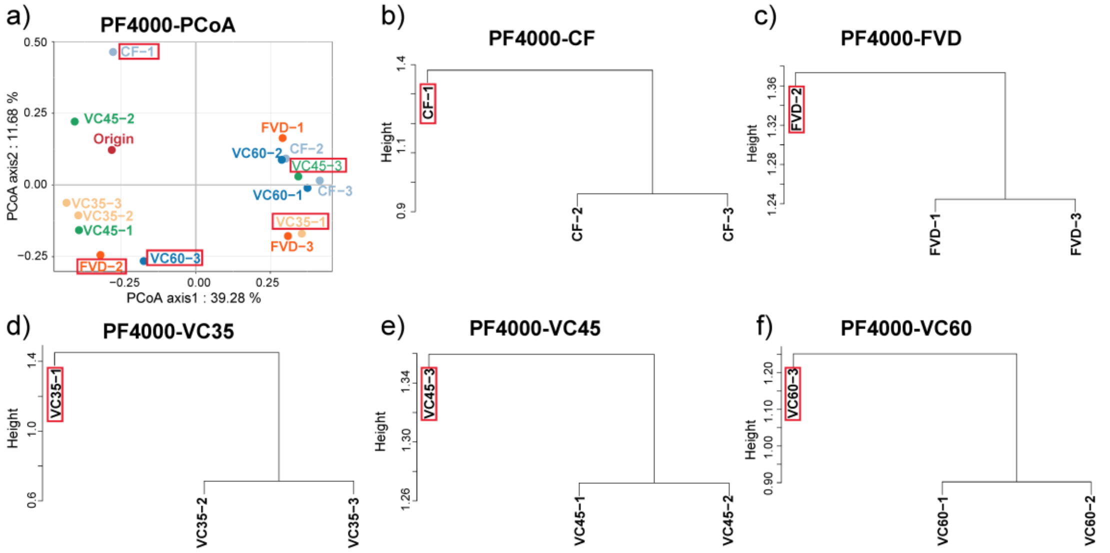


Fig. S6 Replicate screening of PF4000 amplicon samples. (a) PCoA of the samples before screening. (b) – (f) show the clustering relationships of the replicate samples for the CF, FVD, VC35, VC45, and VC60 methods, respectively. The red box line marks things outliner.

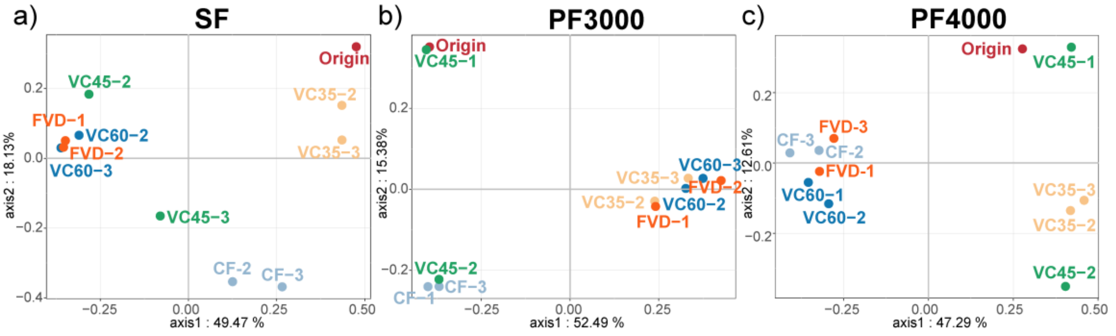
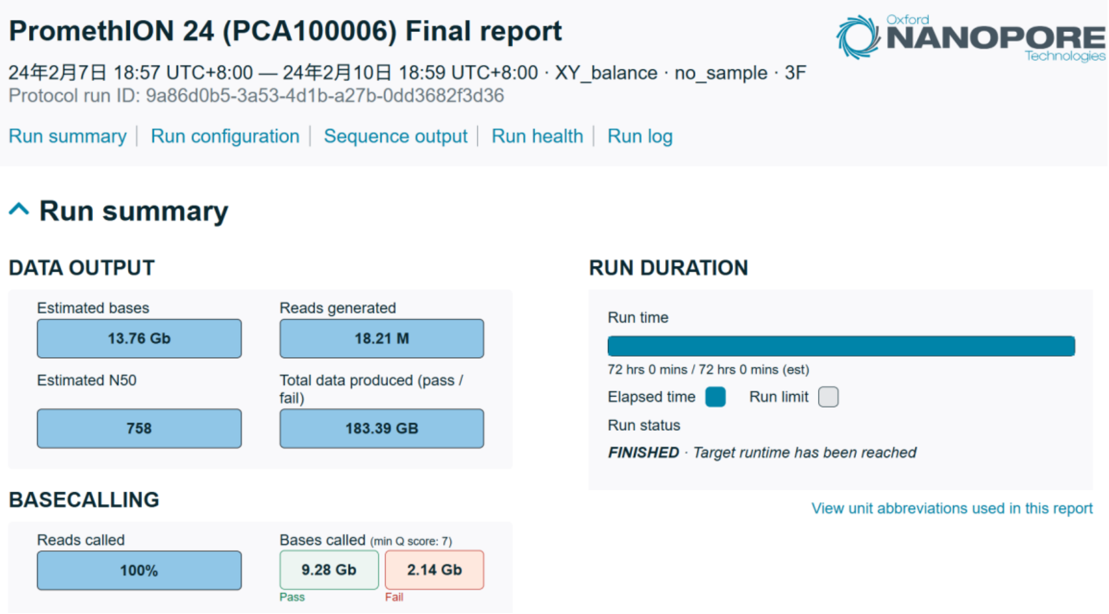


Fig. S7 PCoA diagram of amplicon samples after screening. (a) is the SF sample, (b) is the PF3000 sample, and (c) is the PF4000 sample.



PromethION 24 (PCA100006) Final report

24年2月7日 18:57 UTC+8:00 — 24年2月10日 18:59 UTC+8:00 · XY_balance · no_sample · 3F
Protocol run ID: 9a86d0b5-3a53-4d1b-a27b-0dd3682f3d36

Run summary

DATA OUTPUT

Estimated bases: 13.76 Gb
Reads generated: 18.21 M
Estimated N50: 758
Total data produced (pass / fail): 183.39 GB

RUN DURATION

Run time
72 hrs 0 mins / 72 hrs 0 mins (est)
Elapsed time ■ Run limit ☐
Run status
FINISHED · Target runtime has been reached

BASECALLING

Reads called: 100%
Bases called (min Q score: 7): 9.28 Gb Pass; 2.14 Gb Fail

Fig. S8 Summary of the results of data from nanopore sequencing.

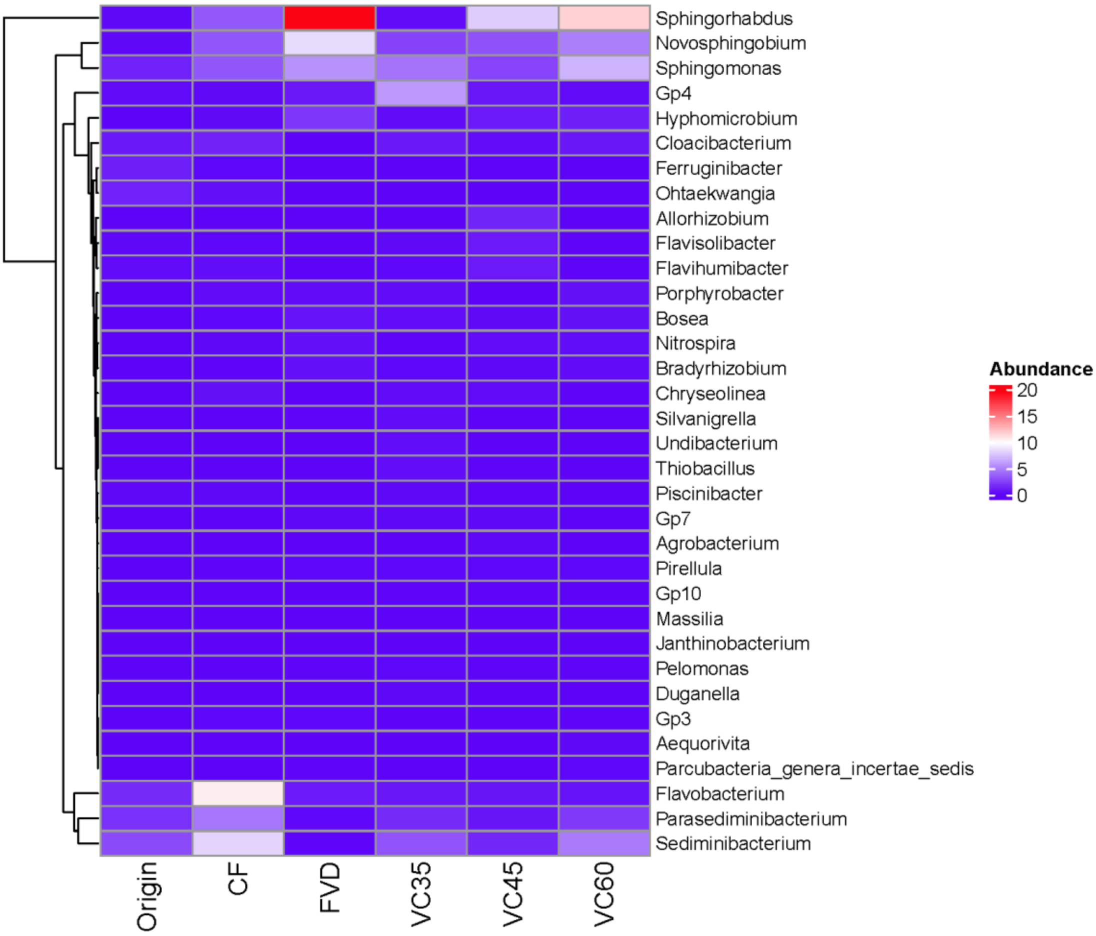


Fig. S9 Variation of community composition based on 16S rRNA amplicon sequencing analysis at the genus level in SF samples before and after enrichment.

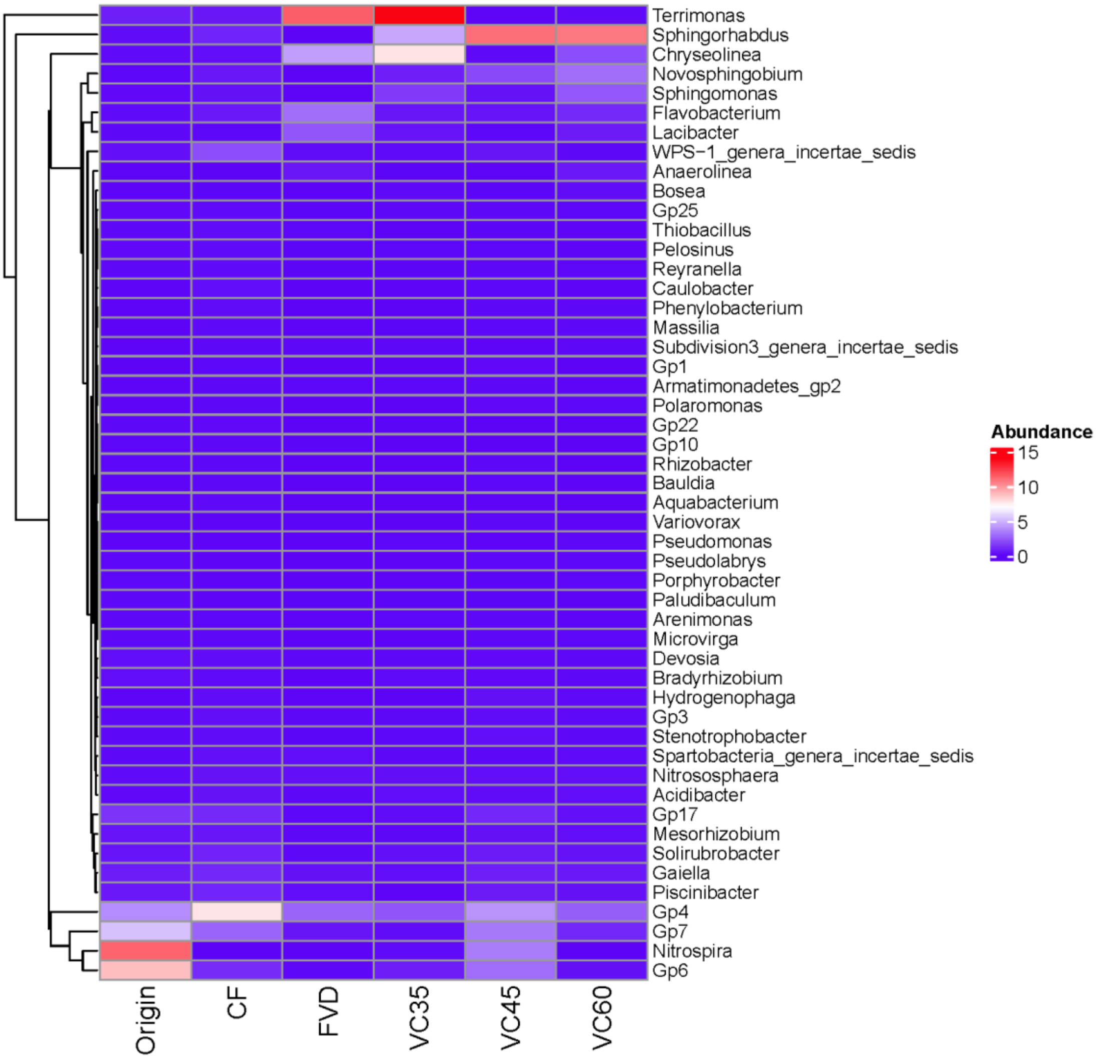


Fig. S10 Variation of community composition based on 16S rRNA amplicon sequencing analysis at the genus level in PF3000 samples before and after enrichment.

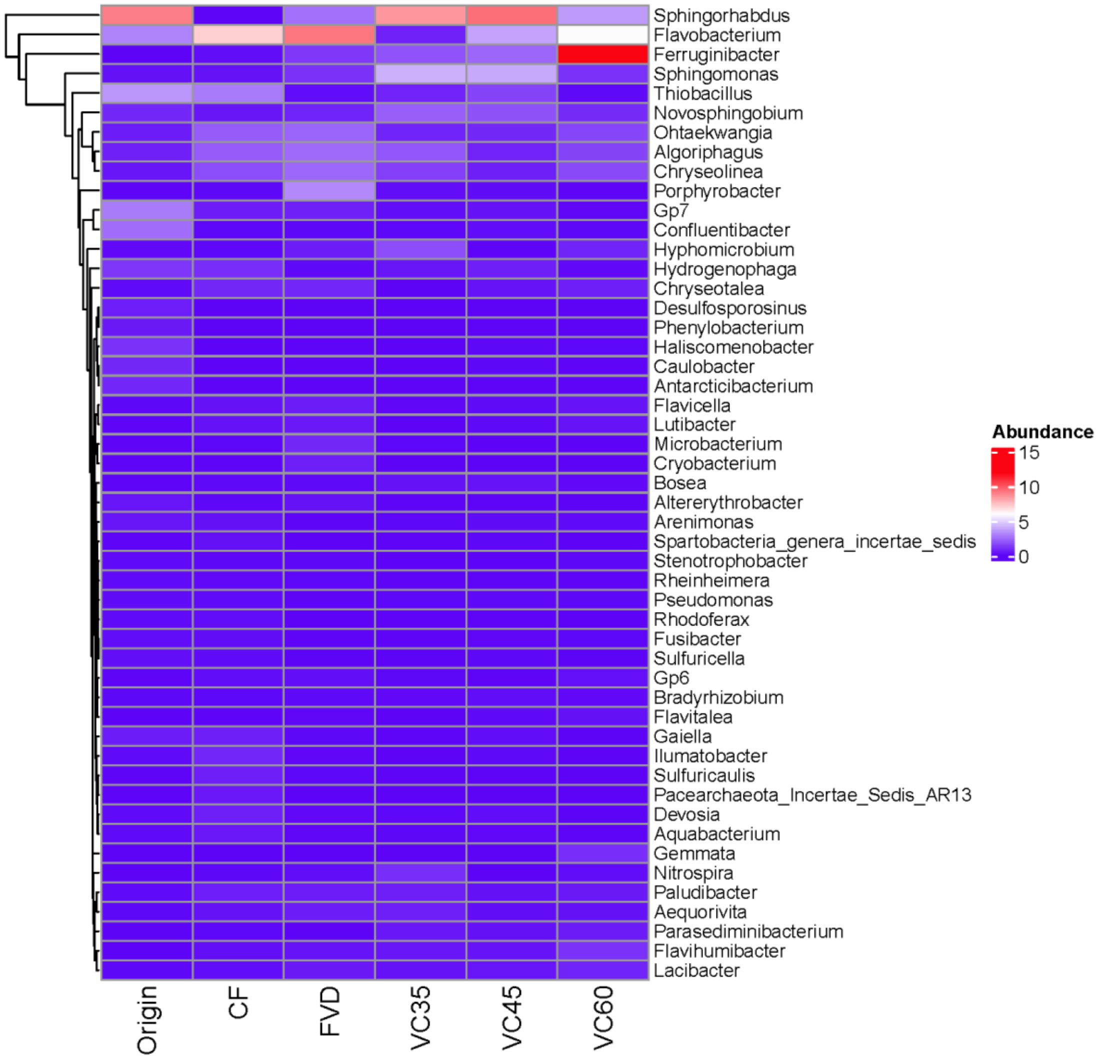


Fig. S11 Variation of community composition based on 16S rRNA amplicon sequencing analysis at the genus level in PF4000 samples before and after enrichment.

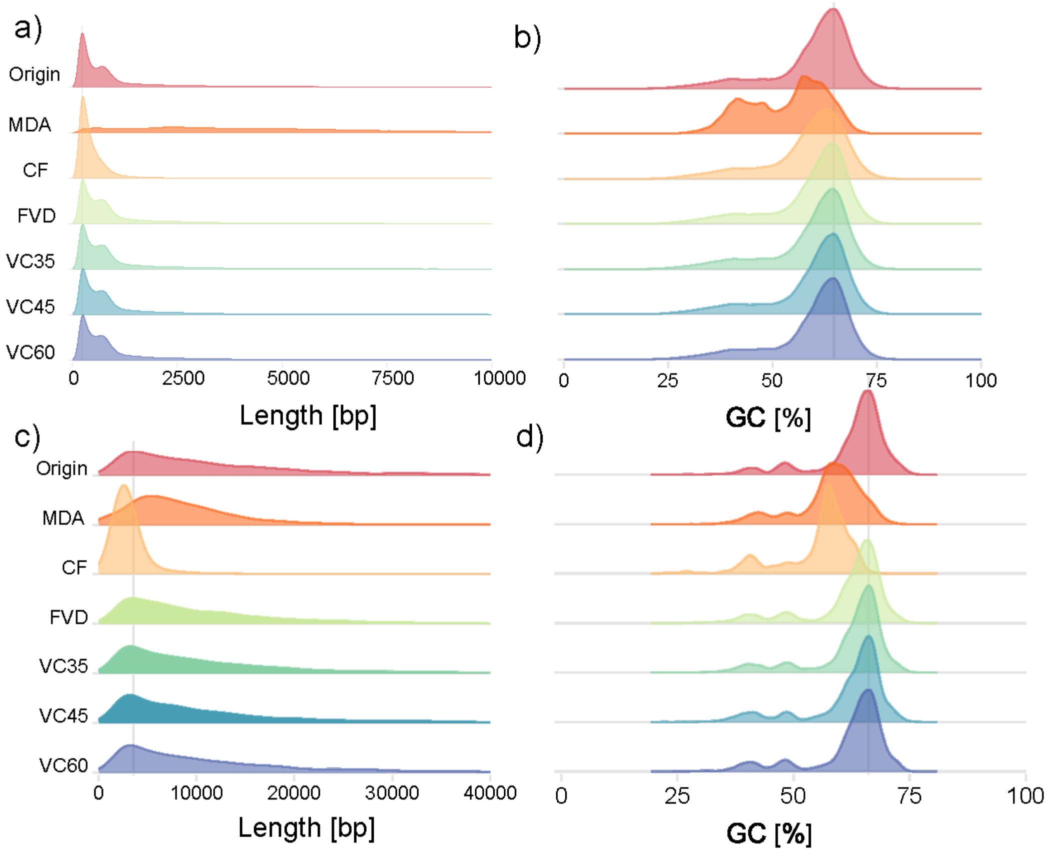


Fig. S12 Length and GC distribution of nanopore reads and assembled contigs. (a) is the length distribution of the nanopore reads, (b) is the GC distribution of the nanopore reads, (c) is the length distribution of the assembled contigs, and (d) is the GC distribution of the assembled contigs.

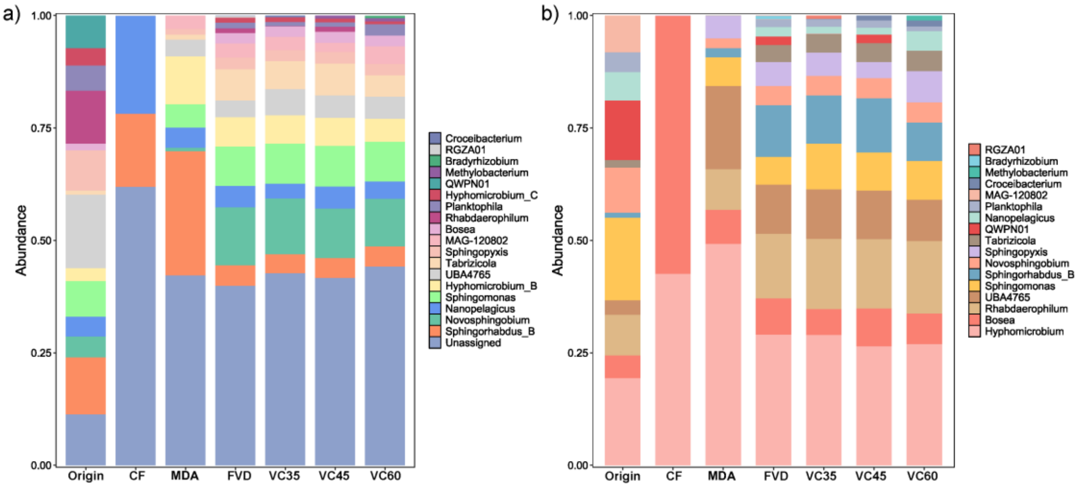


Fig. S13 Genus level community composition of nanopore reads and assembly contigs. (a) is the genus abundance of the reads, and (b) is the genus abundance of the assembled contigs.

Table S1 The concentration and purity of the DNA sample.

	SF			PF3000			PF4000		
Methods	Con (ng/μL)	A260/A280	A260/A230	Con (ng/μL)	A260/A280	A260/A230	Con (ng/μL)	A260/A280	A260/A230
Origin	1.26	1.663	0.032	0.091	1.824	0.371	2.06	1.703	0.155
Origin	1.60	1.597	0.035	0.126	1.816	0.354	2.98	1.728	0.164
CF	3.14	1.916	0.028	0.132	1.872	0.394	7.68	1.760	1.267
CF	3.20	1.766	0.039	0.136	1.877	0.329	7.62	1.698	0.967
CF	3.48	1.841	0.046	0.118	1.877	0.360	7.74	1.758	1.277
FVD	4.20	1.947	0.043	0.172	1.885	0.393	11.60	1.783	0.136
FVD	5.02	1.844	0.043	0.172	1.879	0.403	9.08	1.800	0.127
FVD	4.36	1.793	0.045	0.306	1.888	0.355	9.38	1.778	0.124
VC35	4.14	1.685	0.045	0.208	1.874	0.356	9.20	1.856	0.130
VC35	4.06	1.707	0.046	0.216	1.868	0.359	9.50	1.839	0.101
VC35	4.02	1.651	0.051	0.282	1.862	0.347	9.30	1.817	0.163
VC45	4.08	1.756	0.044	0.164	1.899	0.585	7.52	1.844	0.136
VC45	4.26	1.760	0.043	0.226	1.875	0.298	7.56	1.848	0.127
VC45	4.42	1.732	0.042	0.208	1.868	0.256	7.98	1.853	0.080
VC60	3.12	1.876	1.167	0.386	1.874	1.970	7.82	1.739	0.180
VC60	4.80	2.154	1.401	0.140	1.866	1.953	8.82	1.840	0.150
VC60	2.80	1.812	0.843	0.284	1.861	2.113	8.78	1.857	0.136

Table S2 The absolute quantitative results of qPCR for the samples, with the positive control using activated sludge sample.

Samples	16S rRNA copies/μL	16S rRNA copies/g sample
Positive control	1.60×10^6	1.03×10^8
SF	2.81×10^4	4.31×10^5
PF3000	4.08×10^3	6.47×10^4
PF4000	2.56×10^4	5.81×10^5

Table S3 The data volume after sequencing was calculated by fasta file after polished.

Samples	Methods	Size (GB)	Sample	Methods	Size (GB)
PF4000	Origin	3.90	SF	Origin	2.60
	CF	0.21		CF	9.00
	FVD	0.16		FVD	6.00
	VC35	0.17		VC35	6.40
	VC45	0.15		VC45	6.60
	VC60	0.18		VC60	5.10

Table S4 The total number of bases and reads for each barcode during nanopore sequencing detected are displayed in table below. Reads/bases must have a quality score above 9 to pass.

Samples	Barcode numbers	Methods	Total bases (Mb)	Passed bases (%)	Total reads (k)	Passed reads (%)
SF	barcode13	Origin	2363.47	94.60	668.51	95.60
	barcode14	CF	5483.24	96.10	5261.32	96.30
	barcode15	FVD	5461.5	94.00	1761.03	94.70
	barcode16	VC35	5717.29	95.20	1811.17	96.30
	barcode17	VC45	5967.77	94.10	1989.71	94.90
	barcode18	VC60	4674.78	92.50	1551.56	9.30
PF4000	barcode19	Origin	3743.21	93.70	906.54	94.90
	barcode20	CF	190.50	97.10	63.88	96.10
	barcode21	FVD	142.18	96.70	48.48	95.20
	barcode22	VC35	145.58	96.50	49.37	95.40
	barcode23	VC45	130.43	96.00	42.16	94.10
	barcode24	VC60	156.75	96.00	53.33	95.40

Table S5 Basic sequence information of SF nanopore reads for subsequent analysis. All statistics are based on contigs of size >= 500 bp. Red is worse and blue is better.

Statistics without reference	**Origin**	**MDA**	**CF**	**FVD**	**VC35**	**VC45**	**VC60**
# contigs	354106	212428	452226	429688	426984	433736	430541
# contigs (>= 0 bp)	626847	220916	1539961	698622	696489	722639	710159
# contigs (>= 1000 bp)	192295	199418	91973	216802	216206	215006	211640
# contigs (>= 5000 bp)	54047	92441	932	46045	46052	43377	44742
# contigs (>= 10000 bp)	18920	27106	229	13011	14477	13361	13511
# contigs (>= 25000 bp)	225	1705	39	129	166	131	132
# contigs (>= 50000 bp)	5	41	10	6	4	8	7
Largest contig	244373	80116	117267	75465	181496	101623	89619
Total length	941009552	1189901846	389768598	937246852	946231735	924254662	924225508
Total length (>= 0 bp)	1017110489	1192733077	705558550	1016054119	1024638724	1009206302	1006544211
Total length (>= 1000 bp)	823157330	1180296591	144286207	782909884	793262052	765843086	765901274
Total length (>= 5000 bp)	507757275	862178887	9054455	405327247	417538832	391255312	401543903
Total length (>= 10000 bp)	257203539	410087441	4323196	174062083	196081846	180836139	181686356
Total length (>= 25000 bp)	6922408	53813538	1709107	3931090	5128218	4335315	4214592
Total length (>= 50000 bp)	484249	2403529	753679	366837	367590	595414	483996
N50	5678	7488	838	4039	4122	3871	4014
N90	881	2917	555	795	800	784	784
L50	47064	48635	147627	60142	58317	59518	58286
L90	217702	145618	378201	285460	282059	290293	286872
GC (%)	59.98	55	55.59	59.09	59.17	59.09	59.22

Table S6 Basic sequence information of SF sample nanopore assembly contigs. All statistics are based on contigs of size >= 500 bp. Red is worse and blue is better.

Statistics without reference	**Origin**	**MDA**	**CF**	**FVD**	**VC35**	**VC45**	**VC60**
Raw data (Gb)	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Assembly data （Mb）	59	34	2.7	54	53	54	54
# contigs	2616	3959	838	2994	2840	2815	2868
# contigs (>= 0 bp)	2617	3963	838	2994	2840	2819	2870
# contigs (>= 1000 bp)	2597	3809	838	2979	2823	2797	2851
# contigs (>= 5000 bp)	1952	2842	74	2171	1997	1977	2033
# contigs (>= 10000 bp)	1321	1278	22	1387	1302	1270	1319
# contigs (>= 25000 bp)	517	84	1	463	465	487	475
# contigs (>= 50000 bp)	219	1	0	176	185	180	182
Largest contig	2527710	53169	26432	2190477	2542181	1215984	1970346
Total length	60677741	34418571	2753615	55047294	55113089	53921400	54723651
Total length (>= 0 bp)	60678063	34420074	2753615	55047294	55113089	53923068	54724425
Total length (>= 1000 bp)	60663758	34311881	2753615	55036383	55100684	53907786	54711366
Total length (>= 5000 bp)	58605348	30866221	701588	52458272	52493146	51363228	52137912
Total length (>= 10000 bp)	53963247	19497554	349096	46816828	47360161	46188379	46904181
Total length (>= 25000 bp)	41299184	2630634	26432	32479267	34311753	33999829	33717766
Total length (>= 50000 bp)	30587949	53169	0	22582147	24597950	23244615	23753966
N50	51205	10870	2788	35698	40545	40140	38676
N90	9401	4949	2279	7526	7993	7830	7933
L50	215	1059	272	294	252	264	266
L90	1388	2865	715	1702	1551	1535	1581
GC (%)	62.78	57.84	54.99	62.03	62.2	62.09	62.22

Table S7 MAGs information of medium quality and above obtained from SF samples through binning.

MAGs	Domain	Phylum	Class	Order	Family	Genus	Species	GC(%)	Genome size (Mb)	Completeness	Contamination
Origin_0	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus_B		0.57	3.06	1.30	100.00
Origin_1	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis		0.64	2.60	1.20	91.64
Origin_10	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		0.65	2.03	1.45	56.81
Origin_2	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium_B		0.61	3.18	1.48	92.60
Origin_3	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	JANXVY01		0.67	3.09	1.87	70.44
Origin_4	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	UBA4765	UBA4765	UBA4765 sp002365175	0.61	2.85	3.15	61.77
Origin_5	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		0.66	3.79	0.82	56.59
Origin_6	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae			0.64	2.16	0.84	54.55
Origin_7	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea		0.63	3.35	2.61	76.58
Origin_9	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		0.65	2.89	4.38	62.38
VC35_0	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus_B		0.57	3.06	0.99	99.98
VC35_1	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis		0.64	2.45	0.28	86.84
VC35_12	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		0.65	2.41	0.60	53.28
VC35_2	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium_B		0.61	3.14	1.19	85.54
VC35_3	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		0.67	3.02	2.44	52.44
VC35_5	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	UBA4765	UBA4765	UBA4765 sp002365175	0.61	2.75	0.44	54.60
VC35_7	Bacteria	Actinomycetota	Actinomycetes	Nanopelagicales	Nanopelagicaceae	Nanopelagicus		0.42	1.26	8.42	50.13
VC35_8	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium		0.65	2.45	4.09	50.82
VC45_0	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus_B		0.57	3.05	2.96	100.00
VC45_1	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium_B		0.61	3.15	1.08	89.83

VC45_2	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis		0.64	2.51	0.35	85.42
VC45_3	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	JANXVY01		0.67	2.81	1.72	59.18
VC45_4	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	UBA4765	UBA4765	UBA4765 sp002365175	0.61	3.07	3.89	50.79
VC45_7	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		0.65	2.11	4.61	57.21
VC45_8	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae			0.64	1.79	3.79	50.37
VC60_0	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus_B		0.57	3.00	0.82	100.00
VC60_1	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis		0.64	2.66	0.83	99.78
VC60_11	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Bosea		0.63	1.96	0.06	61.32
VC60_2	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium_B		0.61	2.76	0.15	78.82
VC60_3	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	UBA4765	UBA4765	UBA4765 sp002365175	0.61	2.98	3.14	59.45
VC60_4	Bacteria	Actinomycetota	Actinomycetes	Nanopelagicales	Nanopelagicaceae	Nanopelagicus		0.42	1.24	4.38	52.72
FVD_0	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus_B		0.57	3.08	1.48	100.00
FVD_1	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingopyxis		0.64	2.58	0.61	93.55
FVD_2	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium_B		0.61	3.09	1.03	88.17
FVD_3	Bacteria	Actinomycetota	Actinomycetes	Nanopelagicales	Nanopelagicaceae	Nanopelagicus		0.42	1.35	9.62	75.97
FVD_4	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	JANXVY01		0.67	2.96	1.62	58.15
FVD_6	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas		0.65	2.98	3.43	59.89
FVD_7	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	UBA4765	UBA4765	UBA4765 sp002365175	0.61	3.04	5.16	51.61