

Supplement File S1

Supplemental Tables and Figures

Table S1. Target pathogen and specimen information for the sixteen samples utilized for evaluating the performance of Illumina MiSeq, Ion Torrent PGM and Ion Torrent S5 sequencers.

Sample Name	Target Pathogen	Ct Value	Sample Type	Clinical Specimen Type
EV-D68-1	Enterovirus D68	19.9	Clinical Specimen	Nasopharyngeal swab
EV-D68-2	Enterovirus D68	21.6	Clinical Specimen	Nasopharyngeal swab
EV-D68-3	Enterovirus D68	17.1	Clinical Specimen	Nasopharyngeal swab
EV-D68-4	Enterovirus D68	20.8	Clinical Specimen	Nasopharyngeal swab
Polio-5	Poliovirus 1	N/A	Cell Culture Isolate from Stool Specimen	N/A
Polio-6	Poliovirus 1	N/A	Cell Culture Isolate from Stool Specimen	N/A
Polio-7	Poliovirus 3	N/A	Cell Culture Isolate from Stool Specimen	N/A
Polio-8	Poliovirus 3	N/A	Cell Culture Isolate from Stool Specimen	N/A
Noro-9	Norovirus GII.4-GII.P16	18	Clinical Specimen	Stool
Noro-10	Norovirus GII.4-GII.P16	22	Clinical Specimen	Stool
Noro-11	Norovirus GII.4-GII.P16	27	Clinical Specimen	Stool
Noro-12	Norovirus GII.4-GII.P16	23	Clinical Specimen	Stool
Sapo-13; Parecho-13	Sapovirus II; Parechovirus 1	20.4; N/A	Clinical Specimen	Stool
Sapo-14; Parecho-14	Sapovirus I; Parechovirus 4	22.4; N/A	Clinical Specimen	Stool
Sapo-15	Sapovirus II	21	Clinical Specimen	Stool
Sapo-16	Sapovirus I	18.9	Clinical Specimen	Stool

Table S2. Length of the reference consensus sequence used to compare the results for a given sample across datasets (used for reference-based recruitment and coverage analysis).

Sample	Length (bp)	% GC	Notes
*EV-D68-1	7292	41.5	For analysis, used GenBank Ref- KT995548
*EV-D68-2	7292	41.5	For analysis, used GenBank Ref- KT995535
*EV-D68-3	7292	41.5	For analysis, used GenBank Ref- KT995533
*EV-D68-4	7292	41.6	For analysis, used GenBank Ref- KT995530
Polio-5	7434	46.1	
Polio-6	7444	46.1	
Polio-7	7417	46.3	
Polio-8	7419	46.2	
Noro-9	7546	48.7	Used master consensus for analysis, but there is a previous record in GenBank for this sample/virus (KY947550, length 7555 bp)
**Noro-10	7537	48.6	
**Noro-11	7536	48.6	
**Noro-12	7521	48.6	
Parecho-13	7289	40.1	
Sapo-13	7453	52.0	Used master consensus for analysis, but there is a previous record in GenBank for this sample/virus (MG012419, length 7439 bp)
**Parecho-14	7294	40.1	
Sapo-14	7471	48.7	Used master consensus for analysis, but there is a previous record in GenBank for this sample/virus (MG012440, length 7449 bp)
Sapo-15	7489	52.2	
*Sapo-16	7429	50.9	For analysis, used GenBank Ref- KP298674

*Samples where the closest complete genome in GenBank was used as the reference sequence for comparison.

** Samples where the “master” consensus was longer than any individual de novo assembly consensus sequence in the eight datasets compared.

Table S3. General data output statistics for the Illumina MiSeq, Ion Torrent PGM, and Ion Torrent S5 sequencing platforms.

	Illumina MiSeq*			Ion Torrent PGM**			Ion S5/ S5 XL**				
Sequencing kit/chip	Nano 500v2	500v2	600v3	314v2	316v2	318v2	Ion 510	Ion 520	Ion 530	Ion 540	Ion 550 ^{&}
Expected No. of Reads	1 M	12–15 M	22–25M	0.4–0.55 M	2–3 M	4–5.5 M	2–3 M	4–6 M	9–20 M	60–80 M	100–130 M
Expected Output	500 Mb	7.5–8.5 Gb	13.2–15 Gb	30–100 Mb	300 Mb–1 Gb	600 Mb–2 Gb	0.3–1 Gb	0.6–2 Gb	1.5–8 Gb	10–15 Gb	20–25 Gb
Expected Run Time	24 Hr	~ 39 Hr	~ 56 Hr	2–4 Hr	3–5 Hr	4–7 Hr	2.5–4 Hr				
Data Output Files	FASTQ [§]			BAM, FASTQ, SFF, VCF			BAM, FASTQ, SFF, VCF				
Sequencing mechanism	Sequencing by synthesis			Semi-conductor sequencing chemistry			Semi-conductor sequencing chemistry				
Max Capacity per Run	1 cartridge			2 chips			2 chips				
Read Length	2 x 251 bp		2 x 301 bp	~ 200 bp; 400 bp			~ 200 bp; 400 bp	~ 200 bp; 400 bp; 600bp		~ 200 bp;	~ 200 bp;
Data analysis	On instrument			Off instrument			Off instrument				
Display Interface	Touchscreen			Touchscreen			Touchscreen				

*The expected output of single reads passing filter (unpaired reads).

** Estimated output based on sequencing of a single chip.

[&]The Ion 550 chip is only compatible with the Ion S5 XL, or the Ion GeneStudio S5 Plus or Prime systems.

[§]Further analyses and output formats available using Illumina BaseSpace are not considered in this instance.

Table S4. Number of reads per sample, before and after quality control filtering, for the eight datasets analyzed. The number of raw reads, filtered reads, and proportion (%) of reads remaining after filtering is reported for each sample. The total number of reads, average number of reads per sample, and the standard deviation per sample is shown at the bottom of the table for each dataset.

	PD6			MKN			PD8			SDG		
Sample	Raw	Filtered	%	Raw	Filtered	%	Raw	Filtered	%	Raw	Filtered	%
EV-D68-1	9,355	120	1.3	55,418	545	1.0	27,034	247	0.9	231,320	962	0.4
EV-D68-2	5,151	238	4.6	74,600	4,859	6.5	15,502	808	5.2	117,333	13,305	11.3
EV-D68-3	11,959	126	1.1	70,808	1,042	1.5	30,037	243	0.8	133,394	940	0.7
EV-D68-4	8,739	97	1.1	91,158	1,230	1.3	17,697	195	1.1	112,497	1,059	0.9
Polio-5	13,318	5,301	39.8	79,140	37,480	47.4	35,326	16,610	47.0	107,727	56,688	52.6
Polio-6	19,241	7,290	37.9	93,532	39,321	42.0	45,657	19,842	43.5	154,511	69,768	45.2
Polio-7	23,016	7,897	34.3	88,114	38,821	44.1	46,251	19,021	41.1	164,635	77,995	47.4
Polio-8	29,482	10,762	36.5	110,042	47,120	42.8	66,404	27,475	41.4	146,846	75,908	51.7
Noro-9	49,850	13,085	26.2	48,970	30,105	61.5	80,401	26,153	32.5	89,523	44,005	49.2
Noro-10	46,149	13,482	29.2	78,552	56,489	71.9	83,498	33,603	40.2	129,033	98,484	76.3
Noro-11	90,087	22,719	25.2	58,214	42,205	72.5	133,071	38,948	29.3	139,880	55,324	39.6
Noro-12	101,445	32,416	32.0	82,520	63,356	76.8	170,369	64,107	37.6	141,355	99,064	70.1
Parecho-13; Sapo-13	69,754	22,259	31.9	75,400	46,607	61.8	136,018	51,849	38.1	176,690	92,198	52.2
Parecho-14; Sapo-14	54,657	17,449	31.9	72,364	41,144	56.9	120,976	41,126	34.0	161,627	84,752	52.4
Sapo-15	72,788	20,440	28.1	99,426	55,760	56.1	126,145	44,295	35.1	121,545	58,112	47.8
Sapo-16	71,657	19,234	26.8	97,084	82,079	84.5	165,233	49,701	30.1	132,792	57,714	43.5
Total	676,648	192,915	28.5	1,275,342	588,163	46.1	1,299,619	434,223	33.4	2,260,708	886,278	39.2
Average	42,291	12,057		79,709	36,760		81,226	27,139		141,294	55,392	
Percent Std. Dev. (%)	74.7	81.3		21.0	65.5		65.9	75.2		23.4	62.4	

	SDS			MNN			MK5			MNS		
Sample	Raw	Filtered	%	Raw	Filtered	%	Raw	Filtered	%	Raw	Filtered	%
EV-D68-1	88,610	368	0.4	197,242	1,719	0.9	685,088	6,704	1.0	2,527,692	19,601	0.8
EV-D68-2	156,232	16,755	10.7	241,894	4,408	1.8	932,156	61,629	6.6	2,985,308	43,826	1.5
EV-D68-3	72,230	470	0.7	193,606	2,059	1.1	888,458	8,765	1.0	2,406,842	18,528	0.8
EV-D68-4	129,201	964	0.7	170,446	2,042	1.2	1,168,336	14,813	1.3	2,230,050	22,618	1.0
Polio-5	177,151	80,531	45.5	186,664	65,250	35.0	1,006,544	232,880	23.1	2,530,382	432,678	17.1
Polio-6	260,613	98,223	37.7	196,616	62,682	31.9	1,186,646	239,181	20.2	2,642,664	403,269	15.3
Polio-7	222,556	95,232	42.8	166,988	58,407	35.0	1,098,588	232,591	21.2	2,170,198	370,925	17.1
Polio-8	168,267	83,867	49.8	160,216	57,853	36.1	1,386,466	295,962	21.3	1,783,122	326,745	18.3
Noro-9	128,580	65,137	50.7	156,544	66,097	42.2	606,210	207,901	34.3	1,984,600	501,772	25.3
Noro-10	150,010	115,724	77.1	176,724	97,580	55.2	992,048	446,143	45.0	2,304,330	735,592	31.9
Noro-11	41,089	23,645	57.5	184,012	92,981	50.5	741,024	327,807	44.2	2,368,416	727,790	30.7
Noro-12	153,590	108,485	70.6	165,942	89,195	53.8	1,055,302	502,329	47.6	2,202,858	691,236	31.4
Parecho-13; Sapo-13	189,477	102,526	54.1	128,358	64,208	50.0	953,548	298,129	31.3	1,716,228	407,479	23.7
Parecho-14; Sapo-14	164,302	86,437	52.6	135,498	62,405	46.1	903,804	269,039	29.8	1,785,704	422,587	23.7
Sapo-15	145,751	75,655	51.9	140,148	63,340	45.2	1,248,352	444,887	35.6	1,838,876	490,456	26.7
Sapo-16	141,204	66,515	47.1	158,034	68,342	43.2	1,193,668	713,363	59.8	1,902,818	523,009	27.5
Total	2,388,863	1,015,858	42.5	2,758,932	858,568	31.1	16,046,238	4,290,089	26.7	35,380,088	6,138,111	17.3
Average	149,304	63,491		172,433	53,661		1,002,890	268,131		2,211,256	383,632	
Percent Std. Dev. (%)	35.9	64.6		16.3	61.0		21.1	72.8		16.3	63.9	

Table S5. Percentage loading, clonal and usable reads for Ion Torrent sequencing runs. The Ion Torrent platform which corresponds to each dataset is noted in parentheses (either Ion Torrent PGM or S5).

Dataset	Loading (%)	Clonal (%)	Usable Reads (%)
PD6 (PGM)	87	46	26
PD8 (PGM)	85	49	32
SDS (S5)	97	59	40
SDG (S5)	97	60	38

Table S6. Adjusted p-values for statistical comparison testing the hypothesis that EV-D68 samples had a greater proportion of host reads compared to cell culture (poliovirus samples) or stool samples (norovirus, sapovirus and sapovirus/parechovirus samples).

virus	_virus	Adj. p value
EV-D68	Norovirus	<0.0001
EV-D68	Poliovirus	<0.0001
EV-D68	Sapovirus	<0.0001
Norovirus	Poliovirus	0.0195
Norovirus	Sapovirus	0.3880
Poliovirus	Sapovirus	0.0001

Table S7. Adjusted p-values for statistical comparison testing the hypothesis that a greater proportion of reads were removed during quality/adaptor trimming for Ion Torrent PGM libraries vs. other library preparation/platforms analyzed.

dataset	library kit	_dataset	_library kit	Adj. p value
PD6	Kapa DNA	MK5	Kapa HyperPrep	<0.0001
PD8	Kapa DNA	MK5	Kapa HyperPrep	<0.0001
PD6	Kapa DNA	MKN	Kapa HyperPrep	<0.0001
PD8	Kapa DNA	MKN	Kapa HyperPrep	<0.0001
PD6	Kapa DNA	MN5	Nextera XT	<0.0001
PD8	Kapa DNA	MN5	Nextera XT	<0.0001
PD6	Kapa DNA	MNN	Nextera XT	<0.0001
PD8	Kapa DNA	MNN	Nextera XT	<0.0001
PD6	Kapa DNA	SDG	Kapa DNA	<0.0001
PD6	Kapa DNA	SDS	Kapa DNA	<0.0001
PD8	Kapa DNA	SDG	Kapa DNA	<0.0001
PD8	Kapa DNA	SDS	Kapa DNA	<0.0001

Table S8. Adjusted p-values for statistical comparison testing the hypothesis that a greater proportion of duplicate reads were identified (and removed) for Illumina 500 V2 sequencing runs (MN5, MK5) compared to Illumina Nano 500V2 (MNN, MKN) and Ion Torrent S5 runs (SDS, SDG).

dataset	_dataset	Adj. p value
MK5	MKN	<0.0001
MK5	MNN	<0.0001
MK5	SDG	<0.0001
MK5	SDS	<0.0001
MN5	MKN	<0.0001
MN5	MNN	<0.0001
MN5	SDG	<0.0001
MN5	SDS	<0.0001

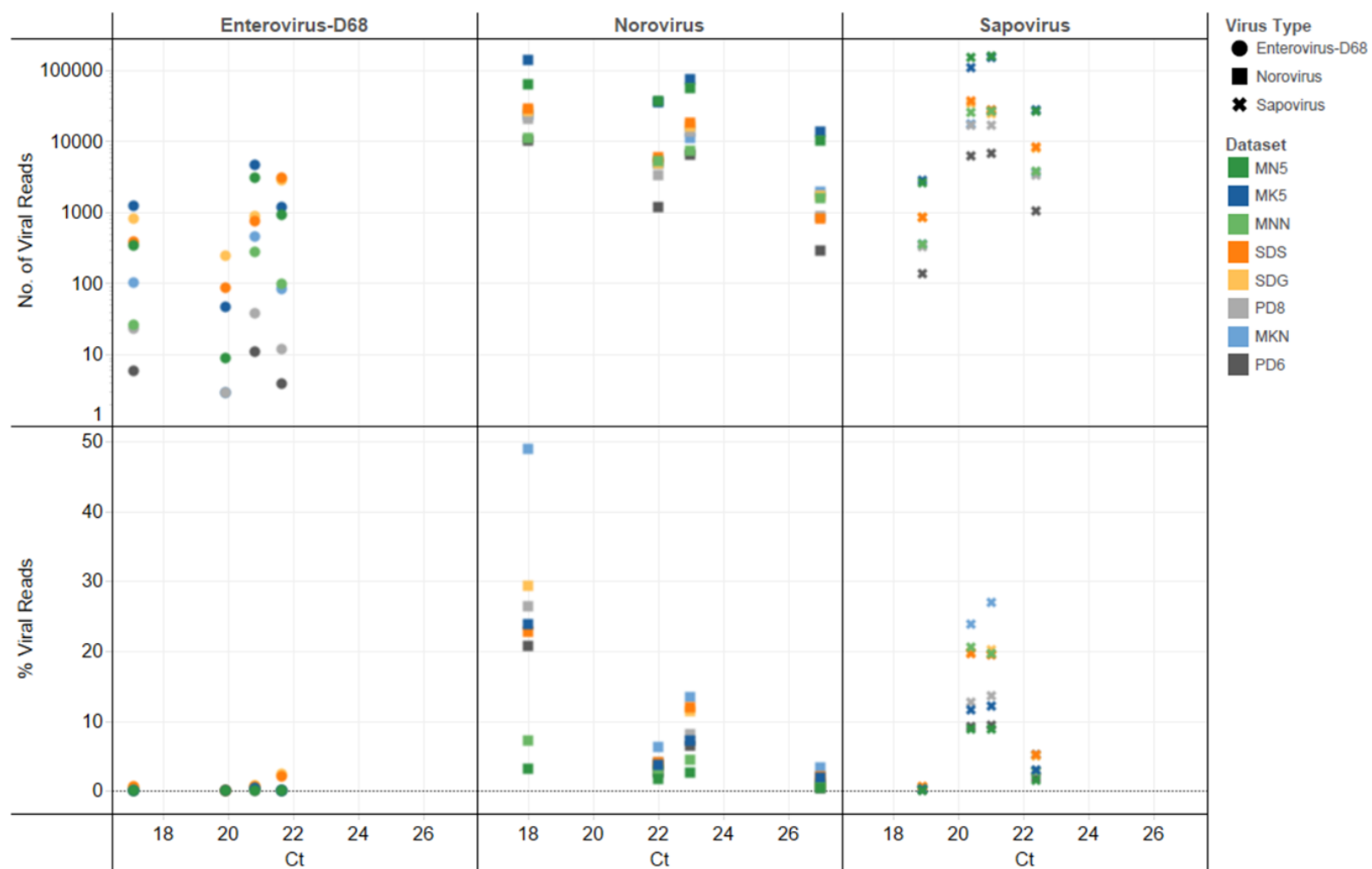


Figure S1. The number of viral reads (top panels) and percentage of target viral reads (bottom panels) as a function of Ct value for EV-D68, Norovirus and Sapovirus samples.

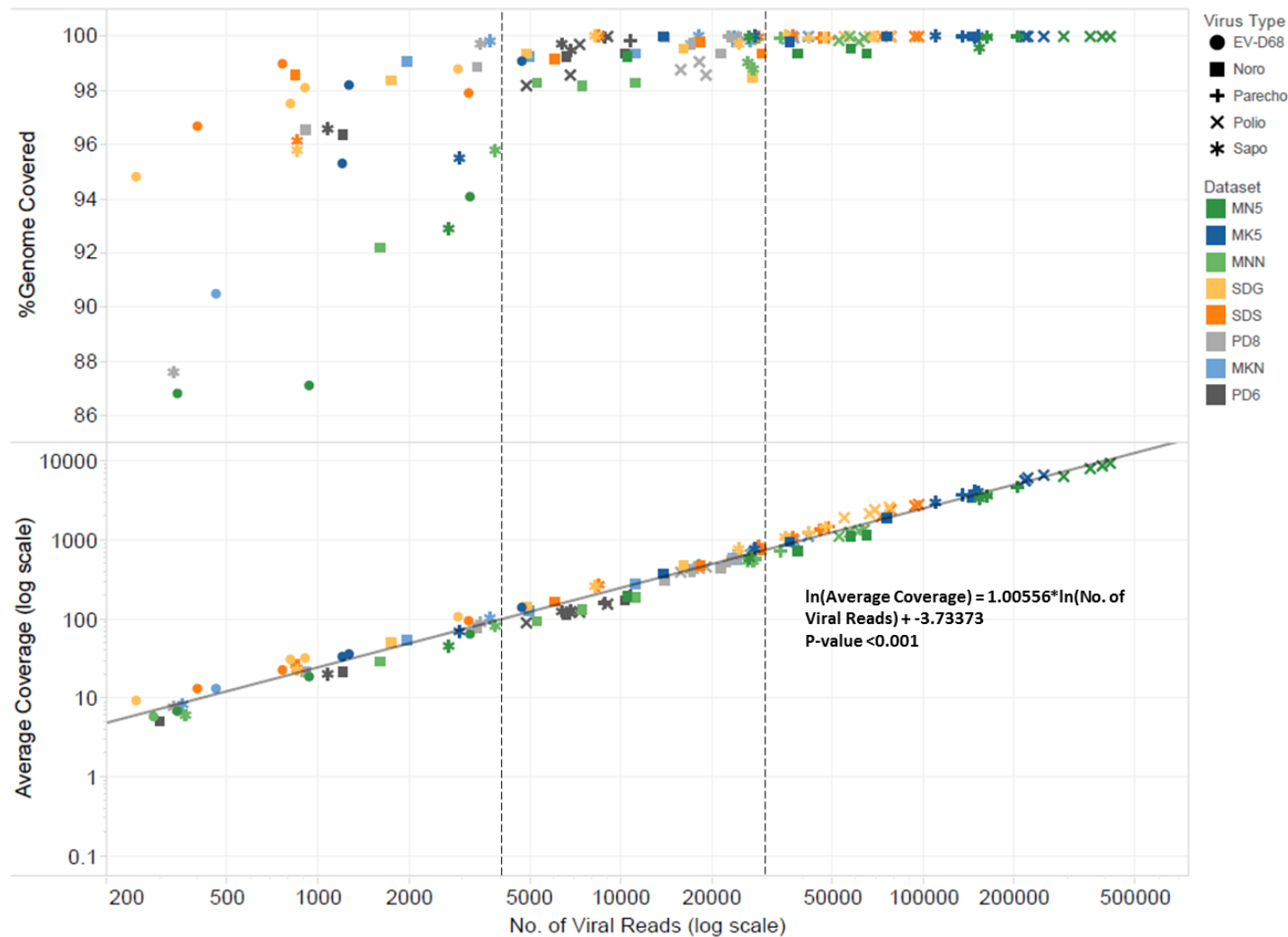


Figure S2. The percentage of the target virus genome with at least 1X read coverage (top panel) and the average read coverage of the genome (bottom panel) as a function of the number of target viral. Each point represents a single sample, with the shape and color of the point corresponding to the target virus and the dataset the sample belonged to, respectively. The dotted line delineates datasets with greater than 4,000 target reads (>98% genome covered), and 30,000 target reads (>99% genome covered).

Table S9. Location and description of indel positions in viral consensus sequences derived from Ion Torrent datasets. Only indels at positions with $\geq 10X$ coverage were considered. Samples EV-D68 1 through 4, and Sapo 16 were not analyzed due to low coverage.

Sample	Position	Dataset(s)	Type of Error	Coverage (Fold)	Sequence
Polio-5	3294	SDS	Indel (Insertion)	2533	CCGCGTCCCCCCCC C GGGCGG
	5155	SDG	Indel (Insertion)	947	CGGCACCCCCCCCC C TGAATG
	5155	SDS		1108	
Polio-6	3306	SDS	Indel (Insertion)	4592	CCGCGTCCCCCCCC C GGGCGG
	5167	SDS	Indel (Insertion)	2443	CGACACCCCCCCCC C TGAATG
Polio-7	4120	SDG	Indel (Insertion)	2438	TTGGCTAAAAAAA A GTTCACG
		SDS		2738	
Polio-8	4123	SDG	Indel (Insertion)	1909	TGGCTCAAAAAA A ATTCACGG
		SDS		1808	
Noro-9	-	-	-	-	-
Noro-10	3965	PD8	Indel (Deletion)	77	AATTCTGGA-TGGTGAGACC
	5727	SDS	Indel (Insertion)	74	TTTCATATTTTT T AGTGCCA
Noro-11	3966	PD8	Indel (Deletion)	22	GAATTCTGGA-TGGTGAGACC
Noro-12	-	-	-	-	-
Parecho-13	598	SDG	Indel (Insertion)	266	CTGGTTAAAAAAA A CGTCTAG
		SDS		371	
	624	SDG	Indel (Insertion)	373	AAACCCGGGGGGGG G ATCCCC
	1556	PD6	Indel (Insertion)	127	AATCTTAAAAAAA A TTCTTA
		PD8		466	
		SDG		1266	
		SDS		1289	
Sapo-13	1831	PD8	Indel (Deletion)	209	GAAACCCCCCCC-GATTTG
		SDG	Indel (Insertion)	777	GAAACCCCCCCCC C GATTTG
		SDS		729	
	3083	PD6	Indel (Deletion)	51	GGTAGGGGGGGG-TGCAAG
		SDG	Indel (Insertion)	973	GGTAGGGGGGGGG G TGCAAG
		SDS		920	
Parecho-14	590	SDG	Indel (Insertion)	766	GTTGGTTAAAAAAA A CGTCTA
		SDS		774	
Sapo-14	4805	PD8	Indel (Deletion)	45	AAACACCAGTTT-CCTGAAAA
Sapo-15	3291	PD8	Indel (Deletion)	94	GCTGCGGACG-CGTGGAGGGT
	4708	PD6	Indel (Deletion)	183	CAGCAACCGC-TCCATCTTTG

Table S10. Estimated cost (total cost and cost per sample) of sequencing preparation by multiplexing level.

The cost for individual procedures used for preparing samples for sequencing was calculated, including pretreatment, SISPA and QC procedures; library preparation; and sequencing chip/cartridge loading. The calculated cost of library preparation also includes the estimated cost of performing the number of SPRI bead cleanups recommended by the kit manufactures.

Pretreatment, Extraction, Random RT and Nucleic Acid Quality Control		Number of Samples Multiplexed					
		1	8	16	24	48	96
Pretreatment (Filtration, Nuclease Treatment) and Extraction	Total Cost	\$ 14.43	\$ 114.00	\$ 227.77	\$ 341.31	\$ 682.14	\$ 1,364.51
	Cost per Sample	\$ 14.43	\$ 14.25	\$ 14.24	\$ 14.22	\$ 14.21	\$ 14.21
Sequence-independent, single primer amplification (SISPA)	Total Cost	\$ 8.77	\$ 58.56	\$ 114.65	\$ 170.73	\$ 339.00	\$ 675.53
	Cost per Sample	\$ 8.77	\$ 7.32	\$ 7.17	\$ 7.11	\$ 7.06	\$ 7.04
QC- SPRI Bead Clean-up (Post-SISPA)	Total Cost	\$ 2.36	\$ 14.22	\$ 28.70	\$ 42.21	\$ 83.00	\$ 164.59
	Cost per Sample	\$ 2.36	\$ 1.78	\$ 1.79	\$ 1.76	\$ 1.73	\$ 1.71
QC- TapeStation (HS D1000 DNA Assay)	Total Cost	\$ 10.63	\$ 44.16	\$ 84.61	\$ 124.00	\$ 250.26	\$ 481.36
	Cost per Sample	\$ 10.63	\$ 5.52	\$ 5.29	\$ 5.17	\$ 5.21	\$ 5.01
QC- TapeStation (D1000 DNA Assay)*	Total Cost	\$ 7.72	\$ 32.13	\$ 59.89	\$ 87.64	\$ 178.99	\$ 340.28
	Cost per Sample	\$ 7.72	\$ 4.02	\$ 3.74	\$ 3.65	\$ 3.73	\$ 3.47
QC- Qubit BR DNA Assay	Total Cost	\$ 3.17	\$ 9.16	\$ 15.97	\$ 23.25	\$ 43.65	\$ 84.47
	Cost per Sample	\$ 3.17	\$ 1.15	\$ 1.00	\$ 0.97	\$ 0.91	\$ 0.88
QC- LabChipGX (quantification/fragment analysis before library pooling)	Total Cost	\$ 7.04	\$ 21.82	\$ 37.83	\$ 53.84	\$ 101.87	\$ 197.92
	Cost per Sample	\$ 7.04	\$ 2.73	\$ 2.36	\$ 2.24	\$ 2.12	\$ 2.06
LIBRARY PREPARATION		Number of Samples Multiplexed					
		1	8	16	24	48	96
NexteraXT (Half Reaction)	Total Cost	\$ 22.70	\$ 173.65	\$ 347.07	\$ 519.53	\$ 1,037.18	\$ 2,072.47
	Cost per Sample	\$ 22.70	\$ 21.71	\$ 21.69	\$ 21.65	\$ 21.61	\$ 21.59
NexteraXT (Full Reaction)*	Total Cost	\$ 39.67	\$ 309.40	\$ 618.59	\$ 926.80	\$ 1,851.71	\$ 3,701.54
	Cost per Sample	\$ 39.67	\$ 38.68	\$ 38.66	\$ 38.62	\$ 38.58	\$ 38.56
Kapa HyperPlus (Half Reaction)	Total Cost	\$ 28.39	\$ 212.21	\$ 424.21	\$ 634.27	\$ 1,265.00	\$ 2,526.48
	Cost per Sample	\$ 28.39	\$ 26.53	\$ 26.51	\$ 26.43	\$ 26.35	\$ 26.32
Kapa HyperPlus (Full Reaction)*	Total Cost	\$ 47.39	\$ 364.80	\$ 729.39	\$ 1,092.04	\$ 2,180.54	\$ 4,357.56
	Cost per Sample	\$ 47.39	\$ 45.60	\$ 45.59	\$ 45.50	\$ 45.43	\$ 45.39
Kapa HyperPrep (Half Reaction)*	Total Cost	\$ 25.39	\$ 188.80	\$ 377.39	\$ 564.04	\$ 1,124.54	\$ 2,245.56
	Cost per Sample	\$ 25.39	\$ 23.60	\$ 23.59	\$ 23.50	\$ 23.43	\$ 23.39
Kapa HyperPrep (Full Reaction)*	Total Cost	\$ 41.39	\$ 316.80	\$ 633.39	\$ 948.04	\$ 1,892.54	\$ 3,781.56
	Cost per Sample	\$ 41.39	\$ 39.60	\$ 39.59	\$ 39.50	\$ 39.43	\$ 39.39
Kapa DNA Library Prep, Ion Torrent Compatible (Half Reaction)	Total Cost	\$ 30.99	\$ 224.23	\$ 448.77	\$ 669.41		
	Cost per Sample	\$ 30.99	\$ 28.03	\$ 28.05	\$ 27.89		
Kapa DNA Library Prep, Ion Torrent Compatible (Full Reaction)*	Total Cost	\$ 46.58	\$ 348.90	\$ 698.10	\$ 1,043.41		
	Cost per Sample	\$ 46.58	\$ 43.61	\$ 43.63	\$ 43.48		

Table S10 continued

SEQUENCING		Number of Samples Multiplexed					
		1	8	16	24	48	96
Sequencing- Illumina MiSeq Nano 500 v2 Kit	Total Cost	\$ 361.41	\$ 362.47	\$ 363.41	\$ 364.34	\$ 367.16	\$ 372.79
	Cost per Sample	\$ 361.41	\$ 45.31	\$ 22.71	\$ 15.18	\$ 7.65	\$ 3.88
Sequencing- Illumina MiSeq 500 v2 Kit	Total Cost	\$ 1,211.41	\$ 1,212.47	\$ 1,213.41	\$1,214.34	\$ 1,217.16	\$ 1,222.79
	Cost per Sample	\$ 1,211.41	\$ 151.56	\$ 75.84	\$ 50.60	\$ 25.36	\$ 12.74
Sequencing- Ion Chef Chip Loading, 316 and 318 chip runs	Total Cost	\$ 398.83	\$ 403.78	\$ 409.44	\$ 415.09		
	Cost per Sample	\$ 398.83	\$ 50.47	\$ 25.59	\$ 17.30		
Sequencing- Ion Chef Chip Loading, 510 and 530 chip runs	Total Cost	\$ 711.96	\$ 716.90	\$ 722.56	\$ 728.21		
	Cost per Sample	\$ 711.96	\$ 89.61	\$ 45.16	\$ 30.34		
Ion PGM 316 Chip	Total Cost	\$ 380.76	\$ 386.71	\$ 393.51	\$ 400.32		
	Cost per Sample	\$ 380.76	\$ 48.34	\$ 24.59	\$ 16.68		
Ion PGM 318 Chip	Total Cost	\$ 620.23	\$ 626.18	\$ 632.98	\$ 639.78		
	Cost per Sample	\$ 620.23	\$ 78.27	\$ 39.56	\$ 26.66		
IonS5 510 Chip	Total Cost	\$ 293.35	\$ 299.30	\$ 306.11	\$ 312.91		
	Cost per Sample	\$ 293.35	\$ 37.41	\$ 19.13	\$ 13.04		
Ion S5 530 Chip	Total Cost	\$ 562.73	\$ 568.68	\$ 575.48	\$ 582.28		
	Cost per Sample	\$ 562.73	\$ 71.08	\$ 35.97	\$ 24.26		

*Indicates procedures NOT considered for calculating the total cost per sample for Figure 5, but merely for comparison to other related methods.



Figure S3. The percentage of the target virus genome with $\geq 10X$ read coverage (top panel) and the average read coverage of the genome (bottom panel) as a function of the number of target viral. Each point represents a single sample, with the shape and color of the point corresponding to the target virus and the dataset the sample belonged to, respectively. The vertical line delineates datasets with greater than 30,000 target reads; with the exception of sample Noro-9 from MN5, greater than 10X coverage across 98% of the genome was observed.