

Supplemental Table 1. Average reads per sample and sequencing analysis summary in dog datasets.

	Total Reads (A)	Remove low quality reads (B)	Collapse to unique reads (C)	Host filter (D)
RNA	11809496	8753321 (74.1%)	1908559 (16.2%)	148828 (1.3%)
DNA	11311839	10258980 (90.7%)	8470962 (74.9%)	359062 (3.2%)
Total	11560668	9506151 (82.2%)	5189760 (44.9%)	253945 (2.2%)

The average number of sequences generated per canine sample was calculated for all RNA-derived and DNA-derived libraries, as well as the total average depth per sample (RNA and DNA together). A) Average number of initial reads. B) Average number of reads remaining after removing low quality sequences. C) Average number of reads remaining after collapsing non-unique sequences into a single read. D) Average number of reads remaining after removing host-derived sequences.

Supplemental Table 2. Positive control sequencing summary.

Sample	Known infectious agent	Raw reads	Reads	
			remaining after host filtering	Number of virus-aligning reads
Green tree python (RNA)	Python nidovirus	8.7×10^6	6.1×10^5 (7.0%)	2077
Mule deer (DNA)	Caprine herpesvirus 2	2.2×10^7	5.0×10^6 (22.4%)	469
American Crow (RNA)	West Nile virus	7.6×10^6	6.2×10^5 (8.2%)	832
American Robin (RNA)	West Nile virus	1.2×10^7	1.9×10^5 (1.5%)	0

Positive control samples that had been previously sequenced using either metagenomic NGS (python and deer) or targeted NGS (crow and robin) were used to validate our sequencing pipeline. Expected results were based on the known viral agents previously detected by sequencing. All expected agents were detected during this metagenomic NGS study, except within the robin, which was later confirmed WNV positive by PCR. The total # of raw reads in each dataset, the number of reads remaining after filtering, and the number of unique individual sequences that aligned to the known agent are provided. NGS, next generation sequencing.