

Table S1. Intrahost Single Nucleotide Variant (iSNV) frequencies. For samples with coverage > 30X, we determined the iSNV frequencies of (F90F; C739T) in the *NP* gene and one transient iSNV (T1186T; T15138C) in the *L* gene. Serum samples underwent unbiased sequencing, PBLs underwent hybrid selection with EBOV-specific baits, and the semen underwent both techniques. We additionally performed single molecule tagged amplicon sequencing to generate higher coverage of the C739T iSNV, (primers listed in Suppl Table 1) which sequences the viral genomic RNA (vRNA) and complementary antigenomic and messenger RNA (c/mRNA) strands independently.

Day	Sample Type	Method	strand	iSNV freq (%)	SE(%)	95% CI
7	Serum	Unbiased	both	10.85%	2.14%	(6.66%, 15.04%)
		Amplicon	c/mRNA	8.21%	0.36%	(7.52%, 8.91%)
		Amplicon	vRNA	6.33%	1.07%	(4.24%, 8.43%)
	PBLs	Hybrid Capture	both	3.72%	1.29%	(1.19%, 6.25%)
		Amplicon	c/mRNA	8.18%	0.39%	(7.42%, 8.95%)
		Amplicon	vRNA	7.60%	0.35%	(6.92%, 8.29%)
8	Serum	Unbiased	both	4.56%	1.11%	(2.38%, 6.74%)
		Amplicon	c/mRNA	8.10%	0.31%	(7.5%, 8.7%)
		Amplicon	vRNA	6.35%	0.37%	(5.63%, 7.07%)
	PBLs	Hybrid Capture	both	6.41%	1.47%	(3.54%, 9.28%)
		Amplicon	c/mRNA	8.06%	0.41%	(7.26%, 8.85%)
		Amplicon	vRNA	7.55%	0.37%	(6.83%, 8.28%)
9	Serum	Unbiased	both	5.21%	1.23%	(2.8%, 7.62%)
		Amplicon	c/mRNA	7.69%	0.31%	(7.08%, 8.29%)
		Amplicon	vRNA	6.04%	0.94%	(4.2%, 7.87%)
	PBLs	Hybrid Capture	both	5.91%	1.73%	(2.52%, 9.3%)
		Amplicon	c/mRNA	7.23%	0.47%	(6.3%, 8.15%)
		Amplicon	vRNA	7.19%	0.34%	(6.54%, 7.85%)
10	Serum	Unbiased	both	5.80%	1.62%	(2.62%, 8.98%)
		Amplicon	c/mRNA	4.62%	0.43%	(3.78%, 5.46%)
		Amplicon	vRNA	6.99%	0.59%	(5.83%, 8.15%)
	PBLs	Hybrid Capture	both	9.76%	4.63%	(0.68%, 18.84%)
		Amplicon	c/mRNA	4.62%	0.43%	(3.78%, 5.46%)
		Amplicon	vRNA	6.99%	0.59%	(5.83%, 8.15%)
32	Semen	Mixed	both	0.72%	0.51%	[0%, 1.72%]
		Amplicon	c/mRNA	3.02%	0.53%	(1.97%, 4.07%)
		Amplicon	vRNA	4.53%	0.73%	(3.11%, 5.96%)
66	Semen	Mixed	both	not enough coverage		
		Amplicon	c/mRNA	0.24%	0.24%	[0%, 0.71%]
		Amplicon	vRNA	0.00%	0.00%	[0%, 0%]
110	Semen	Mixed	both	not enough coverage		
		Amplicon	c/mRNA	not enough coverage		
		Amplicon	vRNA	not enough coverage		
All days	Serum	Unbiased	both	0.00%	0.00%	[0%, 0%]
All days	PBLs	Hybrid Selection	both	[0%, 0%]	[0%, 0%]	[0%, 0%]
32	Semen	Mixed	both	25.56%	3.78%	(18.15%, 32.98%)
66		Mixed	both	[0%, 0%]	[0%, 0%]	[0%, 0%]

Table S2. Primers. Primer sets used and for EBOV detection (orange), human 18s detection (blue) and the stranded amplicon analysis (green and purple).

Name	Sequence (5' to 3')	Design	Reference
F565	TCTGACATGGATTACCACAAGATC	Kulesh NP, anneals to vRNA; 76 bp	13
R640	GGATGACTCTTTGCCGAACAATC	Kulesh NP, anneals to cRNA; 76 bp	13
AG4233_18S1	TCCTTTAACGAGGATCCATTGG	For qPCR of 18S, 101 bp amplicon	6
AG4234_18S1	CGAGCTTTTAACTGCAGCAACT	For qPCR of 18S, 101 bp amplicon	6
RA3v2-UMI-EBOV-739-fwd	TTCTACAGTCCGACGATCNNNNNN GCTTTGTCTTCATCATGCGTACCA	5'-RA3v2-N6-(KJ660346.2 697 to 809)-3'	This Study
RA3v2-UMI-EBOV-739-rev	TTCTACAGTCCGACGATCNNNNNN CTCCATCACGCTTCTTGACTTCA	5'-RA3v2-N6-(KJ660346.2 697 to 809)-3'	This Study
RA5v2-UMI-EBOV-739-fwd	CCTTGGCACCCGAGAATTCANNN NNNGCTTTGTCTTCATCATGCGTAC CA	5'-RA5v2-N6-(KJ660346.2 697 to 809)-3'	This Study
RA5v2-UMI-EBOV-739-rev	CCTTGGCACCCGAGAATTCANNN NNNCTCCATCACGCTTCTTGACTT CA	5'-RA5v2-N6-(KJ660346.2 697 to 809)-3'	This Study
NP-vRNA-fwd	TCATGCGTACCAAGGAGATTAC		This Study
NP-vRNA-probe	TCAAGTATTTGGAAGGGCACGGGT		This Study
NP-vRNA-rev	GCTTCACTCCATCACACTTCT		This Study
NP-cmRNA-fwd	GCGTTAAGCCACAGTTATAGCC		This Study
NP-cmRNA-probe	TGGTAACTCAATATCTTAGCCAGC GA		This Study
NP-cmRNA-rev	GGGCAGGCTAGTAGGTAAGTTAT		This Study