

# Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia

## Technical Appendix 1



**Technical Appendix 1 Figure 1.** Map of Liberia counties showing the 25 Ebola virus (EBOV) isolates described in this study. Samples were collected from persons in 7 coastal Liberian counties (highlighted in green) during September 2014–February 2015.

Ref Pos	Type	Ref Base	Called Base	%EBOV-WA	%EBOV-LIB	Codon	Feature Name	DIAGNOSTICS																
								1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
850	SNP	A	G	100%	100%	G:GGA @ 127 -> G:GGg	NP																	
852	SNP	A	G	100%	100%	K:AAA @ 128 -> R:AgA	NP																	
895	SNP	A	G	100%	100%	T:ACA @ 142 -> T:ACg	NP																	
907	SNP	T	C	1%	0%	N:AAT @ 146 -> N:Ac	NP																	
919	SNP	T	C	100%	100%	F:TtT @ 150 -> F:Ttc	NP																	
1288	SNP	A	T	1%	0%	V:GTA @ 273 -> V:GtI	NP																	
1495	SNP	A	G	100%	100%	Q:CAA @ 342 -> Q:CAG	NP																	
1498	SNP	C	T	1%	4%	L:CTC @ 343 -> L:CTt	NP																	
1507	SNP	T	A	100%	100%	A:GCT @ 346 -> A:Gca	NP																	
1552	SNP	C	T	100%	100%	R:CGG @ 361 -> R:CGt	NP																	
1662	SNP	A	G	100%	100%	S:AGG @ 465 -> G:gGc	GP																	
6396	SNP	T	C	100%	100%	N:AAT @ 466 -> N:Ac	GP																	
6999	SNP	T	A	1%	0%	W:TGG @ 291 -> R:AgG	GP																	
7730	SNP	G	A	100%	100%	E:GAG @ 569 -> E:GAg	GP																	
7775	SNP	A	G	100%	100%	L:CTA @ 579 -> L:CTg	GP																	
7778	SNP	C	A	100%	100%	R:GGG @ 580 -> R:CGa	GP																	
10252	SNP	A	T	1%	4%																			
10253	SNP	A	G	1%	0%																			
12694	SNP	T	A	100%	100%	I:ATT @ 371 -> I:ATA	L																	
12886	SNP	A	C	2%	0%	L:CTA @ 435 -> L:CTc	L																	
12952	SNP	A	G	100%	100%	L:CTA @ 457 -> L:CTg	L																	
13267	SNP	C	T	100%	100%	T:ACC @ 562 -> T:AcI	L																	
13607	SNP	G	A	1%	4%	V:GTC @ 676 -> I:AcI	L																	
13624	SNP	T	G	1%	0	N:AAT @ 681 -> K:AAg	L																	
13630	SNP	A	G	100%	100%	P:CCA @ 683 -> P:CCg	L																	

Mutation has been shown to be tolerated by the diagnostic reagent.

Mutation was previously reported as an untested change (12).

Mutation arose during virus circulation in Liberia 2014-2015.

**Technical Appendix 1 Figure 2.** Mutation analysis of diagnostic binding sites. A single-nucleotide polymorphism (SNP) table is combined with a heat map based on 2 categories: 1) mutations shown to be tolerated by the therapeutic or diagnostic target (highlighted in green); 2) mutations within the binding region of a therapeutic or diagnostic assay that have not yet been tested (highlighted in yellow/orange) (20–24,27,30,31); The column designated “% EBOV-WA” stratifies changes by the percentage of Western African sequences that support each mutation in comparison with EBOV/Kik-9510621. The column designated “% EBOV-LIB” stratifies changes by the percentage of Liberian sequences that support each mutation. Changes previously described are highlighted in yellow; changes that appeared during circulation in Liberia are highlighted in orange. The reference nucleotide positions reported here are in relation to EBOV/Kik (GenBank accession no. AY354458), which is one of the primary isolates used as reference for developing these therapeutic drugs and diagnostic assays. The changes to the probes are summarized in the Technical Appendix 1 Table.

Technical Appendix 1. Diagnostic probe information, used for Figure 2 and Supplementary Figure S4, highlighting mutated probe positions.

Figure references	Author	Gene	Name	Forward	Reverse	Probe	Reference
Fig. 2: Kulesh-MGB	Trombley, A	NP	Kulesh- MGB	TCTGACATGGATTACCACAAGATC	GGATGACTCTTGCCGAACAA <b>T</b> C	AGGTCTGTCCGTTCAA	Am J Trop Med Hyg. 2010 May;82(5):954- 60. doi: 10.4269/ajtmh.2010.09- 0636.
Fig. 2: Kulesh-TM	Trombley, A	GP	Kulesh- TM	TTTCAATCCTAACCGTAAGGC	CAGTCGGTCCCAGAATGTG	GCAGCGATGGG <b>CG</b> CACATG	Am J Trop Med Hyg. 2010 May;82(5):954-60. doi: 10.4269/ajtmh.2010.09-0636.
Fig. S4 #1	Sanchez, A	NP	ZAI-NP	GGACCGCCAAGGT <b>AAAAA</b> TGA	GCATATTGTTGGAGTTGCTTCAGC		J Infect Dis. 1999 Feb;179 Suppl 1:S164- 9.
Fig. S4 #2	Towner, J.	NP	EboZNP	TGG <b>AAA</b> ACATTAAGAGAACACTTGC	AGGAGAG <b>AA</b> ACTGACCGGC <b>A</b> T	CATGCCGAAGAGGAG <b>AC</b> ACTGAAGC	Biosecur. Bioterror. 9, 361- 371 (2011)
Fig. S4 #3	Weidmann , M.	NP	ENZ	ATGATGGA <b>AG</b> CTACGGCG	AGGACCAAGTCATCTGGTGC		J. Clin. Virol. 30, 94-99 (2004).
Fig. S4 #4	Trombley, A	NP	Ebola MGB- EBOV	<b>ACTC</b> AGAGAGGC <b>T</b> GCCACTG	CAAGTCCAAGATGGTCAAGTT <b>C</b> G		Am J Trop Med Hyg. 2010 May;82(5):954- 60. doi: 10.4269/ajtmh.2010.09- 0636.
Fig. S4 #5	Gire, SK	VP24*	KGH	GTCGTTCC <b>AA</b> CAATCGAGCG	CGTCCCGTAGCTTRGCCAT		Science. 2014 Sep 12;345(6202):1369- 72. doi: 10.1126/science.1259657. Epub 2014 Aug 28.
Fig. S4 #6	Sanchez, A	GP	EBO-GP	AATGGGCTGAAA <b>T</b> TGCTACAATC	TTTTTTAGTTCCC <b>AG</b> AAGGCCACT		J Infect Dis. 1999 Feb;179 Suppl 1:S164- 9.
Fig. S4 #7	Gunther, S.	GP	EBOGP	TGGGCTGAAA <b>Y</b> TGCTACAATC	CTTTGTGMACATASC <b>GG</b> CAC	CTACCAGCAGGCCAG ACGG	Antiviral Res. 2004 Sep;63(3):209-15.
Fig. S4 #8	Morvan, J. M.	GP	EBO1/2	TGGGTAA <b>TY</b> ATCCTYTTCCA	ACGACACCTTCAGCRAAAGT		Microbes Infect. 1, 1193- 1201 (1999).
Fig. S4 #9	Strong, J.E.	GP	ZebovG P	GGCCAACGA <b>G</b> ACGACTCAA	AAAGGT <b>GCG</b> TAGCTCAGTTGTG		Proc Natl Acad Sci U S A. 2008 Nov 18;105(46):17982- 7. doi: 10.1073/pnas.0809698105. Epub 2008 Nov 3.
Fig. S4 #10	Morvan, J. M.	GP	EBO3/4	GTTTGTGKGACAAACTGTC	TGGAARGCWAAGTCWCCGG		Microbes Infect. 1, 1193- 1201 (1999).
Fig. S4 #11	Leroy, E	L	modified Filo AB	ATCGGAATTTCTTCATTGAAAGA	ATG <b>T</b> GGTGG <b>G</b> TTATAATAATCACTG <b>A</b> CATGCAT		J Med Virol. 2000 Apr;60(4):463- 7.
Fig. S4 #12	Sanchez, A	L	Filo AB	ATCGGAATTTCTTCATT*	ATG <b>T</b> GGTGG <b>G</b> TTATAATAATCACTG <b>A</b> CATG		J Infect Dis. 1999 Feb;179 Suppl 1:S164- 9.
Fig. S4 #13	Panning, M	L		AAGCATTTCTAGCAATATGATGGT	ATG <b>T</b> GGTGG <b>G</b> TTATAATAATCACTG <b>A</b> CATG	CC <b>AAA</b> ATCATCACT <b>GT</b> GTGGTGCCA	J Infect Dis. 2007 Nov 15;196 Suppl 2:S199-204.
Fig. S4 #14	Palacios, G.	L		<b>AA</b> ACACCGGGCTTAATTCTTATATCAA	GGTGGTAAAATTCCCATAG <b>T</b> AGTTCTTT		Emerg Infect Dis. 2006 Apr;12(4):692- 5.
Fig. S4 #15	Zhai, J.	L		TATTTCCATT <b>T</b> CAAAAACACTGGG	GCTTCACAAAGTGTGAACATT		J. Clin Microbiol. 2007 Jan;45(1):224- 6. Epub 2006 Nov 1.
Fig. S4 #16	Grard, G.	L	PanFilo- L1/2	ATMGRAAYTTTCYTTYCWT	TG <b>W</b> GGHGGRYTATAAWARTCACTD <b>A</b> CAT		J. Infect. Dis. 204 (suppl. 3), S776-S784 (2011).

Figure references	Author	Gene	Name	Forward	Reverse	Probe	Reference
Fig. S4 #17	Grard, G.	L	PanFilo-L3/4	<b>GCNAARGCMTTYCCHAGYAAATGATGG</b>	ATAAWARTCACTDA <b>CATGCATRTARCA</b>		J. Infect. Dis. 204 (suppl. 3), S776–S784 (2011).
Fig. S4 #18		L	GAB-1	<b>GAATGTAGGTAGAACCTTCGG</b>	<b>GCATATAACACTGTGGGATTG</b>		