Electronic Supplementary Information for:

Impact of Primer-Dimers and Self-Amplifying Hairpins on Reverse Transcription Loop-Mediated Isothermal Amplification Detection of Viral RNA

Robert J. Meagher[1] *, Aashish Priye[1], Yooli K. Light[2] Cheng Huang[3], Eryu Wang[4]

1 = Sandia National Laboratories, Biotechnology and Bioengineering Department, PO Box 969, Livermore, CA 94550, USA

2 = Sandia National Laboratories, Systems Biology Department, PO Box 969, Livermore, CA 94550, USA

3 = University of Texas Medical Branch, Department of Pathology, 301 University Blvd, Galveston, TX 77555, USA

4 = University of Texas Medical Branch, Department of Microbiology and Immunology, 301 University Blvd, Galveston, TX 77555, USA

* Corresponding author: rmeaghe@sandia.gov; +1 925 294 6847

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In addition, we have provided an additional supplementary file containing MATLAB and C source files that can be used for calculating $\Delta G'$(NSA). Documentation is included within the code file.

Supplementary Table S1 Complete set of primers for modified YFV assay, with adaptation for QUASR. Underlined sequences within the FIP and BIP primers reflect the F1c and B1c regions of those primers. IBFQ = Iowa Black FQ quencher. The primers are based upon the publication by Kwallah *et al*. [1]

Primer name	Sequence	Concentration (nM)
YFV F3	TCCACACCTTGGAGGCATTA	200
YFV B3	GTCCATCACAGTTGCCATCA	200
YFV FIP Bump -8	GGCCTCCGATTGATCTCGGC TTTT GATTACAAGGAGTGTGAGTGG	1600
YFV BIP	GGTTCAGACGAACGGACCTTGG TTTT CCCTGGGCAAGCTTCTCT	1600
YFV LoopF-Cy3	Cy3-CTTCAACTGATGTTCCAATCGTATG	800
YFV LoopB	ATGCAGGTACCACTAGAAGTGA	800
YFV LFc-12-IBFQ	CATCAGTTGAAG-IBFQ	1200

Supplementary Table S2: Complete set of primers for modified DENV assay. Underlined sequences within the FIP and BIP primers reflect the F1c and B1c regions of those primers. The primers are based upon the publication by Lau *et al*. [2]

Primer name	Sequence	Concentration (nM)
DENV1 F3	TGGGGTAGCAGACTAGTGG	50
DENV1 B3	TCTGTGCCTGGAATGATGC	50
DENV1 FIP	CCACCAGGGTACAGCTTCCC GACCCCTCCCAAAACACAA	400
DENV13 BIP-Cy5 Bump+2	Cy5-AGAGGTTAGAGGAGACCCCCC CAGGATCTCTGGTCTCTCCC	800
DENV1 LoopF	TGGTGTTGGGCCCCGCT	200
DENV13 LoopB Bump+2	AAACAGCATATTGACGCT	400
DENV2 F3	TACGCATGGCGTAGTGGA	50
DENV2 B3	GCGTTCTGTGCCTGGAAT	50
DENV2 FIP Bump-4	TCATCTCACCTTGGGCCCCCC TAGCGGTTAGAGGAGACC	400
DENV24 BIP-Cy5	Cy5-AGAGGTTAGAGGAGACCCCCC GCAGGATCTCTGGTCTTTCC	800
DENV2 LoopF	GTTGCTGCGATTTGTAA	200
DENV2 LoopB	ACAGCATATTGACGCTG	200
DENV3 F3	GCTGTACGCACGGTGTAG	50
DENV3 B3	CCTGGAATGATGCTGAGGAG	50
DENV3 FIP	GGTACAGCTTCCCTCAGTGCTC GTGGTTAGAGGAGACCCCT	400
DENV3 LoopF	CTGCTGCGTTGTGTCAT	200
DENV3 LoopB	CAGCATATTGACGCTGG	200
DENV4 F3	CGCGTGGCATATTGGACTA	50
DENV4 B3*	TGCCTGGATTGATGTTGCA	50
DENV4 FIP Bump+3	CAGCTTCCTCCTGGCTTCG GTTAGAGGAGACCCCTCCCA	400
DENV4 LoopF	CCCCTTTTGCTGCGTTT	200
DENV4 LoopB	AAACAGCATATTGACGC	200
DENV1234 BIPc-10	TCTAACCTCT-IBRQ	2400

*DENV4 B3 sequence reflects a correction described in a comment by the authors on the publisher's site for Lau *et al* [2].

Supplementary Table S3: Thermodynamic parameters for original and modified YFV, DENV2 and DENV4 FIP primers.

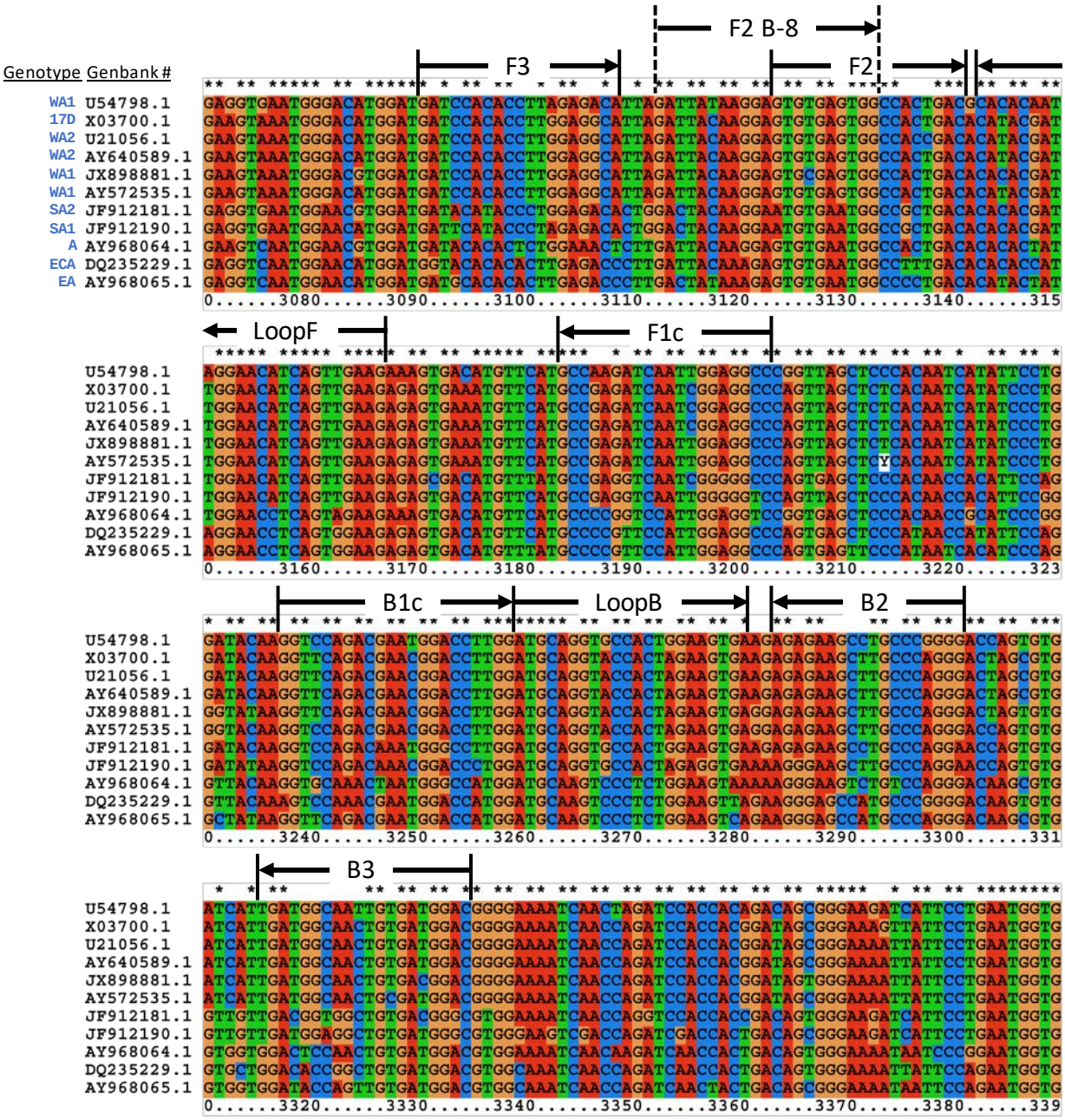
FIP Primers	Max ΔG (Perfect primer binding) (kJ/mol)	Total Hairpin Structures	Amplifiable Hairpin Structures	Total Dimer Structures	Amplifiable Dimer Structures
YFV Original	-85.19	10	4	19	1
YFV Modified	-88.25	7	0	9	1
DENV2 Original	-83.48	10	7	15	4
DENV2 Modified	-81.46	6	1	12	3
DENV4 Original	-83.06	16	10	14	3
DENV4 Modified	-81.33	14	0	6	1

Supplementary Table S4: Comparison of performance for DENV primers (pan-serotype assay) either using originally reported primer sequences or a combined set using the modified DENV2 and DENV4 FIP, for replicate reactions at two concentrations for each serotype of DENV near the expected limit of detection.

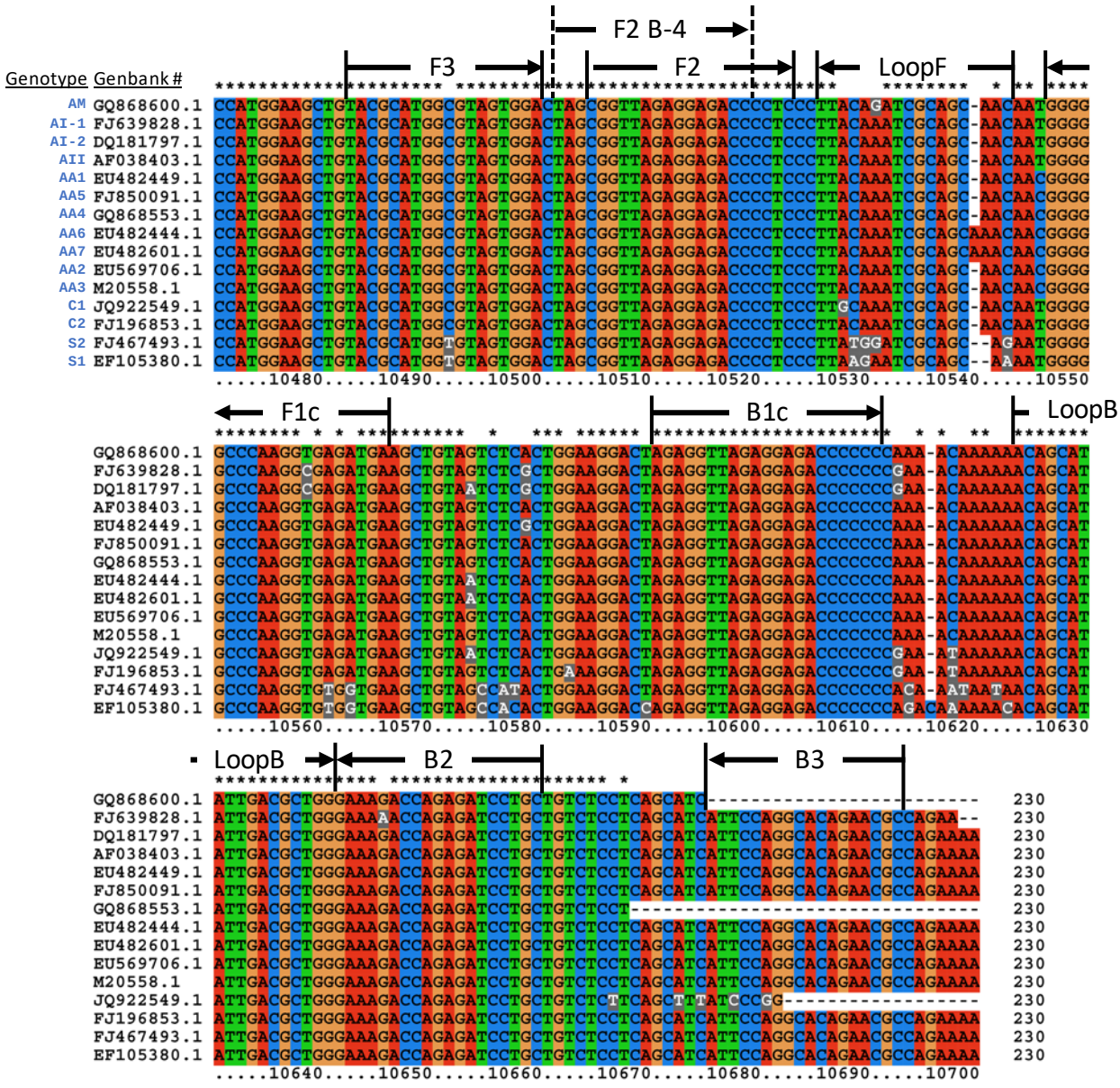
		Original DENV primers		Modified DENV2 and DENV4 FIP	
Target	copies	Number positive	Mean time	Number positive	Mean time
DENV1	200	10 / 10	24 ± 2	10 / 10	22 ± 2
	50	6 / 10	30 ± 8	9 / 10	24 ± 3
DENV2	200	8 / 10	31 ± 4	7 / 10	30 ± 3
	50	3 / 10	33 ± 5	1 / 10	35
DENV3	200	5 / 10	27 ± 3	4 / 10	34 ± 8
	50	2 / 10	31 ± 4	1 / 10	45
DENV4	200	10 / 10	29 ± 8	10 / 10	22 ± 3
	50	3 / 10	37 ± 5	6 /10	28 ± 8

Supplementary Table S5: Limits of detections with 50% and 90% probability, and corresponding confidence intervals for assay targets from the modified YFV and DENV assays. Detection limits are listed as copy number of viral genomic RNA input per 10-µL assay. LOD and confidence intervals were determined from probit analysis, using BioStat software. N refers to the total number of replicates across all concentrations included in the probit analysis, excluding no-template control reactions.

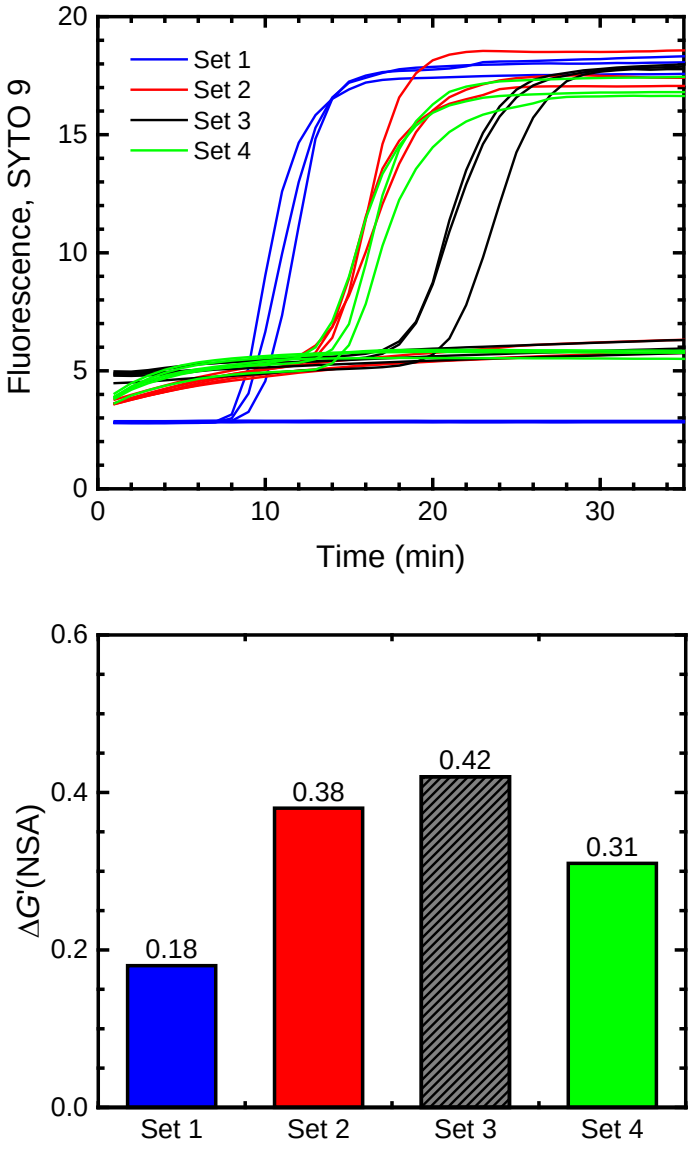
Target	LOD_{50}	LOD_{50} 95% confidence	LOD_{90}	LOD_{90} 95% confidence	N total
YFV RNA	170	120 – 240	910	600 – 1700	168
DENV-1 RNA	8	2 – 12	30	20 – 80	110
DENV-2 RNA	60	50 – 90	160	110 – 310	110
DENV-3 RNA	70	40 – 100	200	120 – 570	68
DENV-4 RNA	50	30 – 80	190	110 – 520	68



Supplementary Figure S1: YFV sequence alignment of a portion of the NS1 gene showing primer binding sites, including original and modified (bumped) F2 region of FIP. Note FIP and BIP primers have a "TTTT" linker between the F1c and F2 (or B1c and B2) regions. Genotypes are labeled as per Beasley *et al* [3]. WA1, WA2 = West Africa 1 & 2; 17D = 17D vaccine strain (likely West African origin), SA1, SA2 = South American 1 & 2; A = Angola; ECA = East-Central African; EA = East African.



Supplementary Figure S2: DENV-2 sequence alignment of 3'-UTR region showing primer binding sites, including original and modified (bumped) F2 region of FIP. Genotypes and sub-genotypes are labeled as per Waman *et al* [4]. AM = American, AI-1, AI-2 = Asian I; AII = Asian II, AA1-AA7 = Asian-American; C1, C2 = Cosmopolitan; S2, S2 = Sylvatic



Supplementary Figure S3: Real-time amplification curves (top) and probability of non-specific amplification ($\Delta G'$(NSA), bottom), calculated for 4 candidate primer sets evaluated for an Ebola RT-LAMP assay (unpublished). The real-time curves at top illustrate 3 positive controls and 3 no-template controls for each primer set. The $\Delta G'$(NSA) at bottom are calculated for the entire primer set.

References (also cited in main text)

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