Kinetics of elementary steps in Loop-mediated isothermal amplification (LAMP) show that strand invasion during initiation is rate-limiting-SI

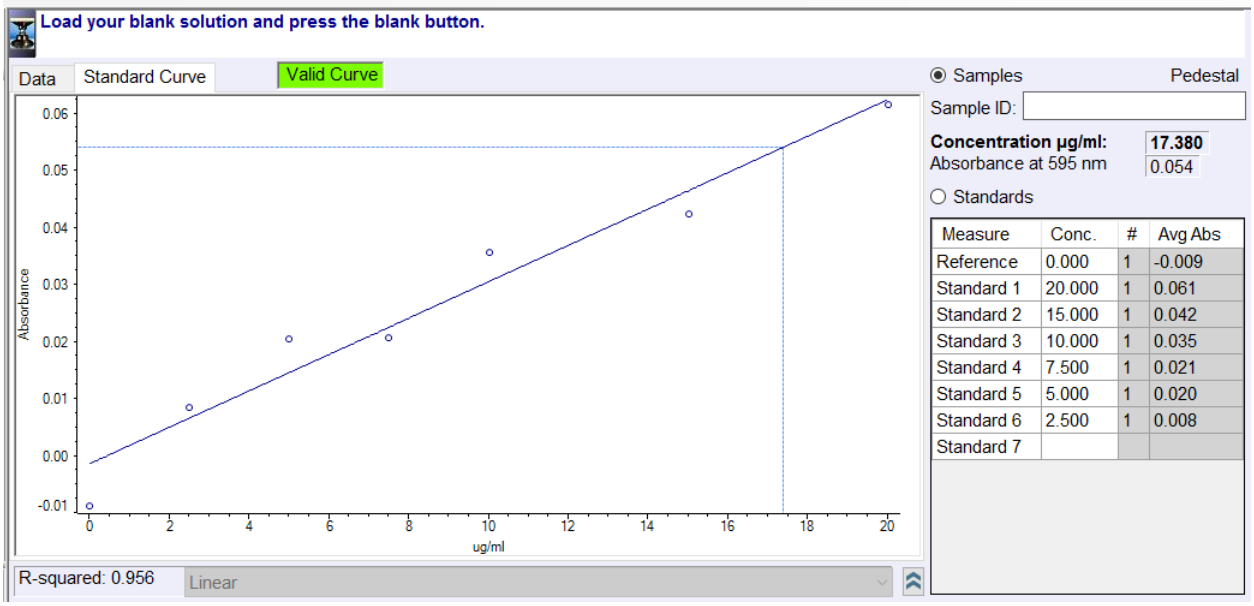

Figure S1: Bradford Assay. The Bradford standard curve was generated using Pierce™ Bovine Serum Albumin Standard Ampules, 2 mg/mL, and Nanodrop measurement. Absorbance at 595 nm was plotted to generate the standard curve shown above. Concentration of unknown protein sample was then calculated from the measured absorbance.

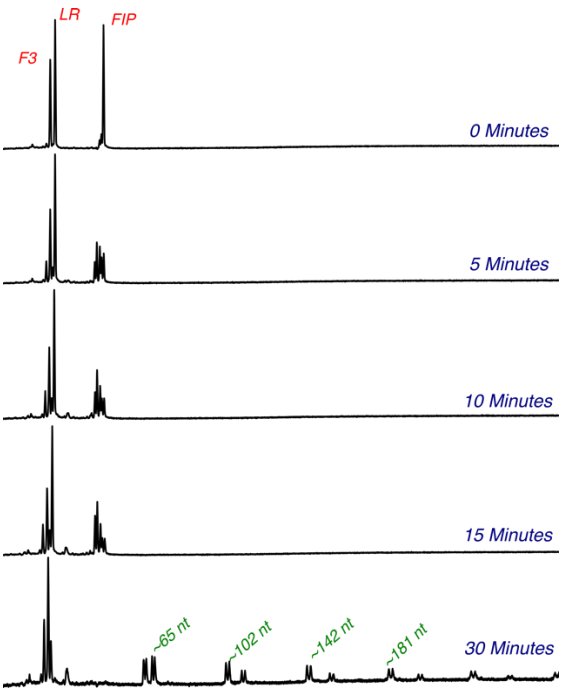

Figure S2: Initiation reaction with GAPDH LAMP primers without added template DNA. Initiation reactions in B-F were performed by mixing a solution of 1 μM each of FAM-F3, FAM-LR, FAM-FIP and 600 nM Bst-LF polymerase with 400 μM dNTPs to start the reaction. Time points were quenched by mixing with EDTA. Time points are given in blue on the right side of each electropherogram. For each electropherogram for each individual time point, retention time is given on the x-axis with increasing retention times from left to right, and fluorescence intensity the y-axis.

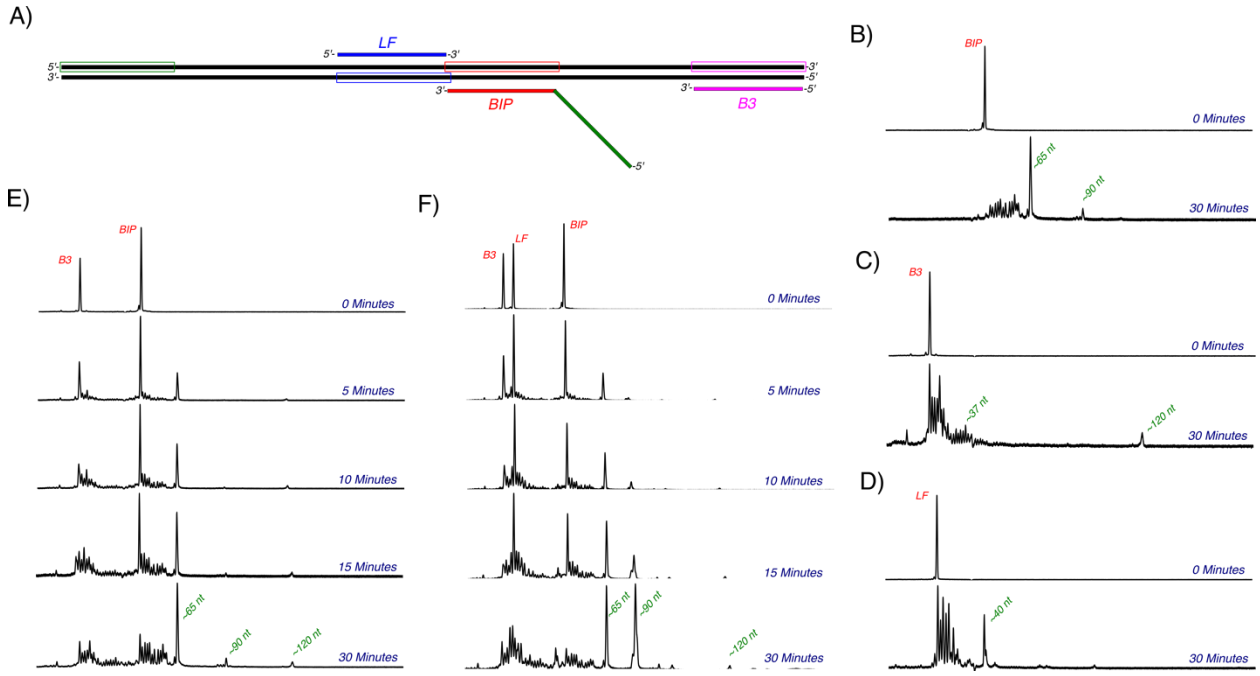

Figure S3: Multiple primers are required for efficient LAMP initiation – *A. aegypti* template. A) Schematic of template DNA and primers used to measure initiation. The strand the primer anneals to is shown in the box around the template DNA (black) in the same color as the corresponding primer. BIP is shown annealed to the red region, however the tail of BIP in green can also anneal to the green boxed region next to the LF annealing region. B) Initiation reaction with template DNA and BIP. For each electropherogram for each individual time point, retention time is given on the x-axis with increasing retention times from left to right, and fluorescence intensity the y-axis. Initiation reactions in B-F were performed by mixing a solution of 1 μM indicated FAM labelled primer(s), 250 nM template DNA LT-1/LT-1C and 600 nM Bst-LF polymerase with 400 μM dNTPs to start the reaction. Time points were quenched by mixing with EDTA. Time points are given in blue on the right side of each electropherogram. Major extension products after 30 minutes are a product at 65 nt and a full-length extension product at 90 nt. C) Initiation reaction with template DNA and B3. Most extension was by a few nucleotides however a minor peak at 120 nt appeared after 30 minutes. D) Initiation reaction with template DNA and LF. Extension products up to 40 nt were observed after a 30-minute incubation. E) Initiation reaction with B3 and BIP. A non-specific amplification product at 65 nt is observed forming after 5 minutes. Extension to full length extension products was observed between 10 and 30 minutes. Sizes of products are given in green next to the corresponding peaks in the electropherogram. F) Initiation reaction with B3, LF, and BIP. Extension to the nonspecific product at 65 nt and the specific product at 90 nt was observed starting at 5 minutes.

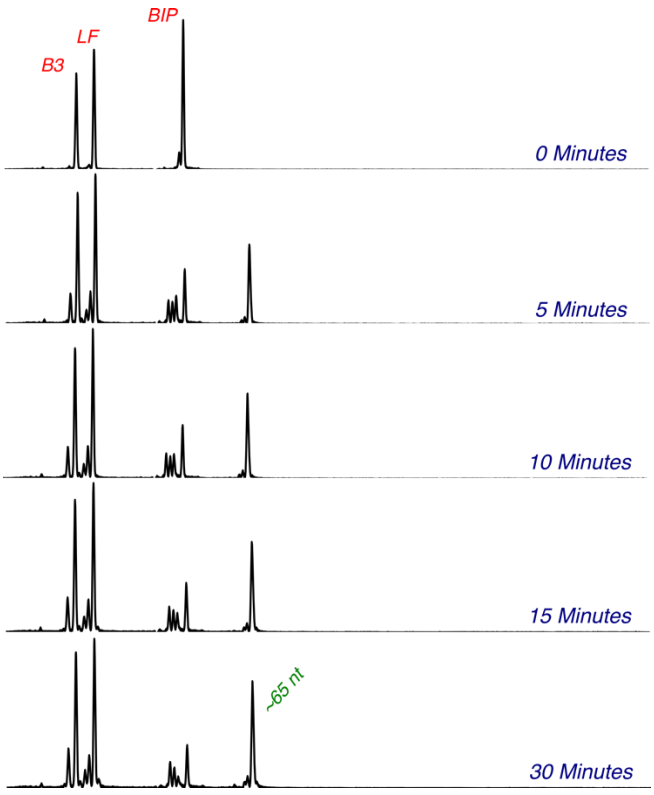

Figure S4: Initiation reaction with *A. aegypti* LAMP primers without added template DNA. The initiation reaction was performed by mixing a solution of 1 μM each of *A. aegypti* FAM-B3, FAM-LF, FAM-BIP and 600 nM Bst-LF polymerase with 400 μM dNTPs to start the reaction. Time points were quenched by mixing with EDTA. Time points are given in blue on the right side of each electropherogram. For each electropherogram for each individual time point, retention time is given on the x-axis with increasing retention times from left to right, and fluorescence intensity the y-axis.

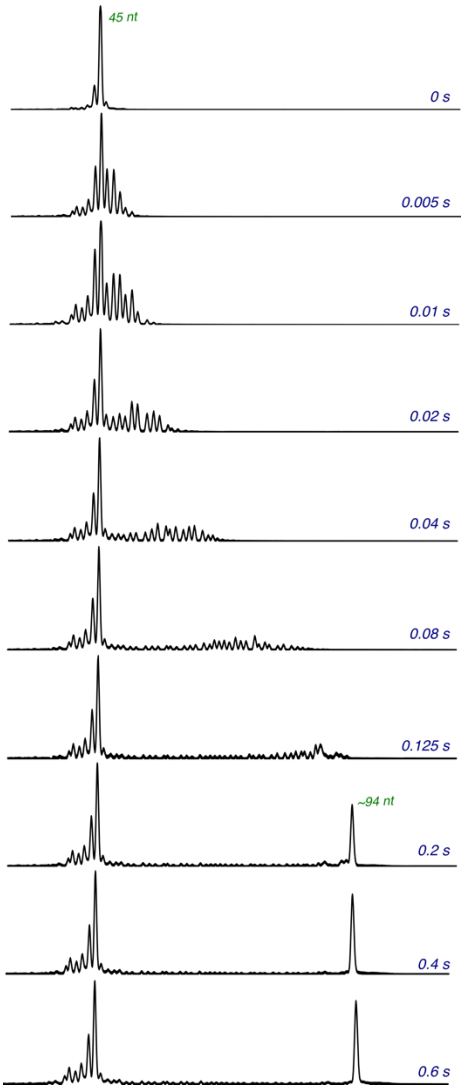

Figure S5: Primer extension on *A. aegypti* linear template. A solution of 75 nM *A. aegypti* FAM-BIP/LT-1 and 400 nM Bst-LF polymerase were mixed with 400 μM dNTPs to start the reaction in the quench flow. Time points were quenched with EDTA and analyzed by capillary electrophoresis. Lengths of products corresponding to major peaks are shown in green. Time points are given on the right-hand side of the electropherograms in blue. For each electropherogram for each individual time point, retention time is given on the x-axis with increasing retention times from left to right, and fluorescence intensity the y-axis. The average rate of polymerization on this template was 300 nt/s.

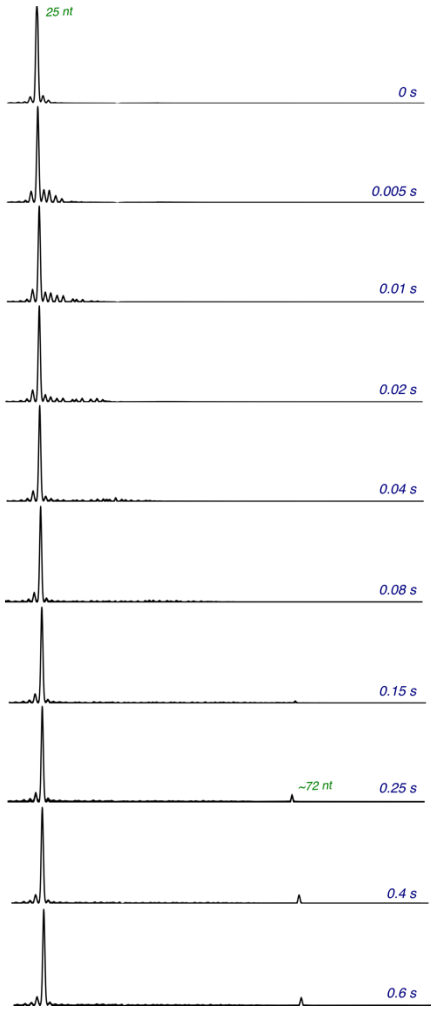

Figure S6: Synthesis from loop primer on *A. aegypti* hairpin template. A solution of 75 nM FAM-BIP/HT-1 and 400 nM Bst-LF polymerase were mixed with 400 μM dNTPs to start the reaction in the quench flow. Time points were quenched with EDTA and analyzed by capillary electrophoresis. Lengths of products corresponding to major peaks are shown in green. Time points are given on the right-hand side of the electropherograms in blue. For each electropherogram for each individual time point, retention time is given on the x-axis with increasing retention times from left to right, and fluorescence intensity the y-axis. The average rate of polymerization on this template was 150 nt/s.

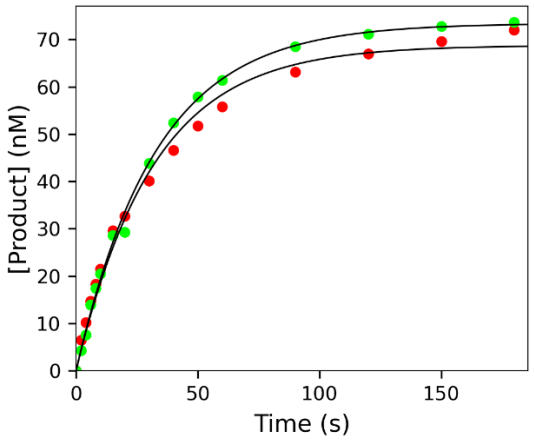

Figure S7: Primer annealing kinetics on hairpin template vs linear template. A solution of 100 nM template DNA (GAPDH HT-1 for hairpin, green; LF-1 for linear template, red) and 400 nM Bst-LF polymerase was mixed with 75 nM FAM-labelled primer (FAM-LR for hairpin, FAM-FIP for linear template) and 400 μM dATP, dCTP, and dTTP to start the reaction. Time points were quenched by mixing with EDTA. The data fit single exponential functions with rates of 0.03 ± 0.002 s^{-1}.