Supplementary Information

Vibration mixing for enhanced paper-based recombinase polymerase amplification

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This Supplementary Information describes:

A

outcome	copies
0	0
0	0
0	0
0	15
0	15
0	15
0	30
1	30
1	30
0	60
1	60
1	60
1	100
1	100
1	100
1	200
1	200
1	200

B

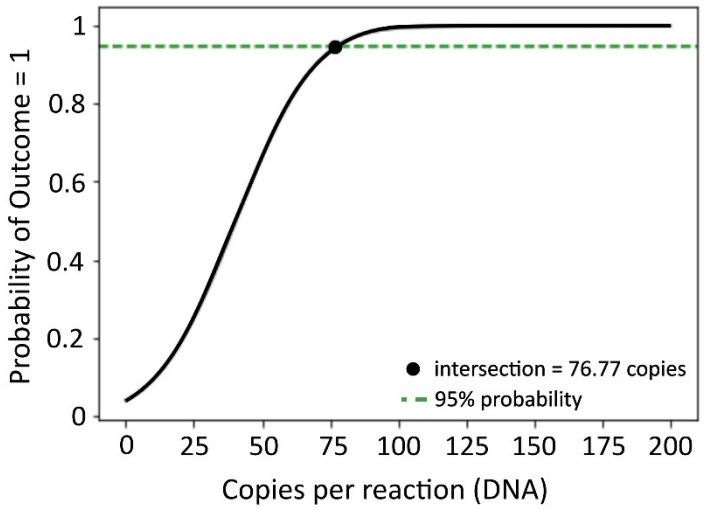

Figure S1. Table A: Binary outcomes for tested copy numbers on unmixed RPA reactions with DNA and MgOAc integrated into RPA reagent and buffer (Fig 4b). 1 represents positive result and 0 represents negative result. B: Probit model results. At a 95% probability of success, the LoD is approximately 77 copies per reaction of DNA for unmixed reactions in Figure 4b.

To determine the limit of detection for the unmixed reaction in Figure 4, we used a probit analysis to find the approximate copy number that corresponds to a 95% outcome probability. For both 30 and 60 copy reactions, only two of the three experiments were positive reactions. All positive and negative reactions are based on the overall bulk fluorescence with the threshold set as the average of the NTCs plus 3 standard deviations. Based on the probit model, the LoD is around 77 copies per reaction for HIV DNA.

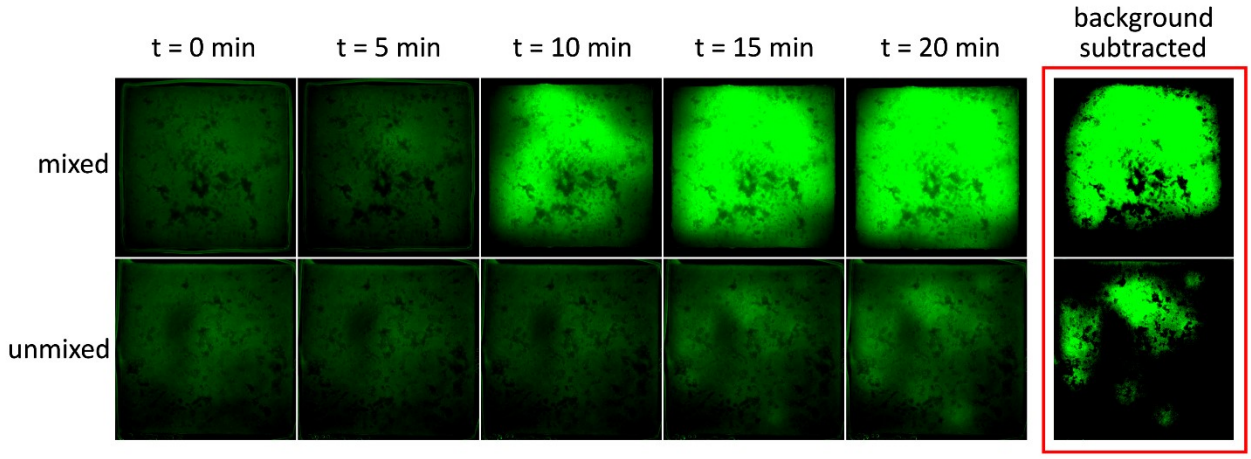

Figure S2. Top row: Images of progression of mixed, *homogenized,* RPA from 0-20 minutes. Bottom row: Image of progression of unmixed, *homogenized*, RPA from 0-20 minutes.

Figure S2 displays the image progression of mixed and unmixed homogenized RPA experiments. The images for t = 20 minutes are also seen in Figure 4A.

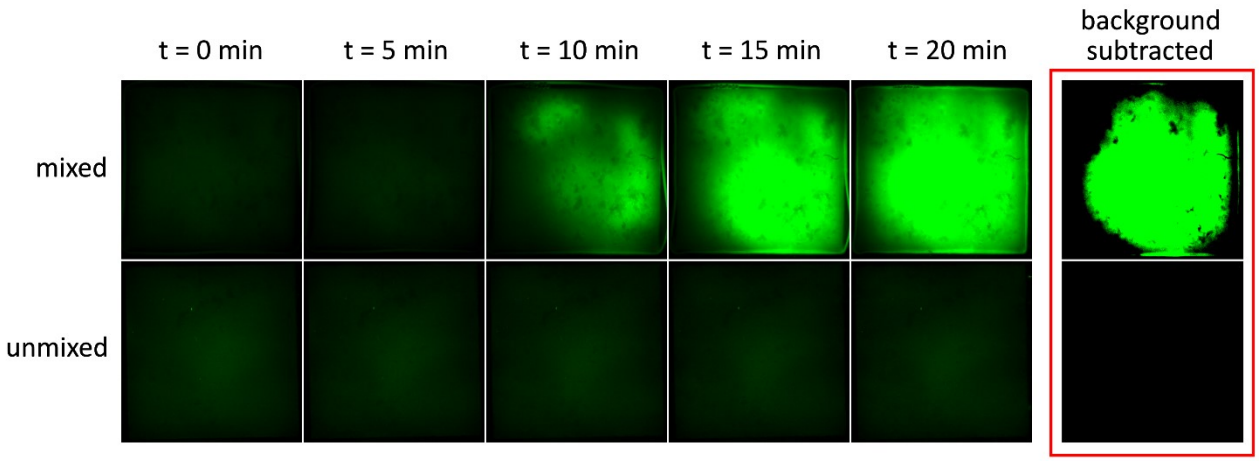

Figure S3. Top row: Images of progression of mixed, *isolated*, target and magnesium RPA from 0-20 minutes. Bottom row: Image of progression of unmixed, isolated, target and magnesium RPA from 0-20 minutes.

Figure S3 displays the image progression of mixed and unmixed separated target and magnesium RPA experiments. The images for t = 20 minutes are also seen in Figure 6A.

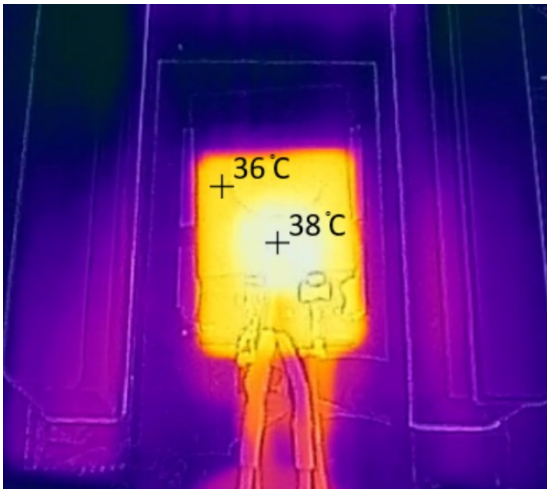

Figure S4. FLIR image of the motor and PTC on with a 2 V and 4 V input respectively. With the motor on, the motor face increases to 38 C while the PTC remains at 36 C.

Figure S4 depicts the temperature profile of the motor and PTC on. With a 2V input to the motor, the motor face increases from 36 C to 38 C. The surrounding area of the PTC remains at 36 C and provides additional area to maintain RPA temperature and improve temperature stability over time. Without the PTC, only the motor area is heated to 38 C. The motor only assembly is more susceptible to temperature fluctuations and requires a slightly longer heat up time to reach RPA temperatures. These temperatures were confirmed using a thermocouple (80BK-A, Fluke Networks, USA) attached to the surface of the motor face and PTC.

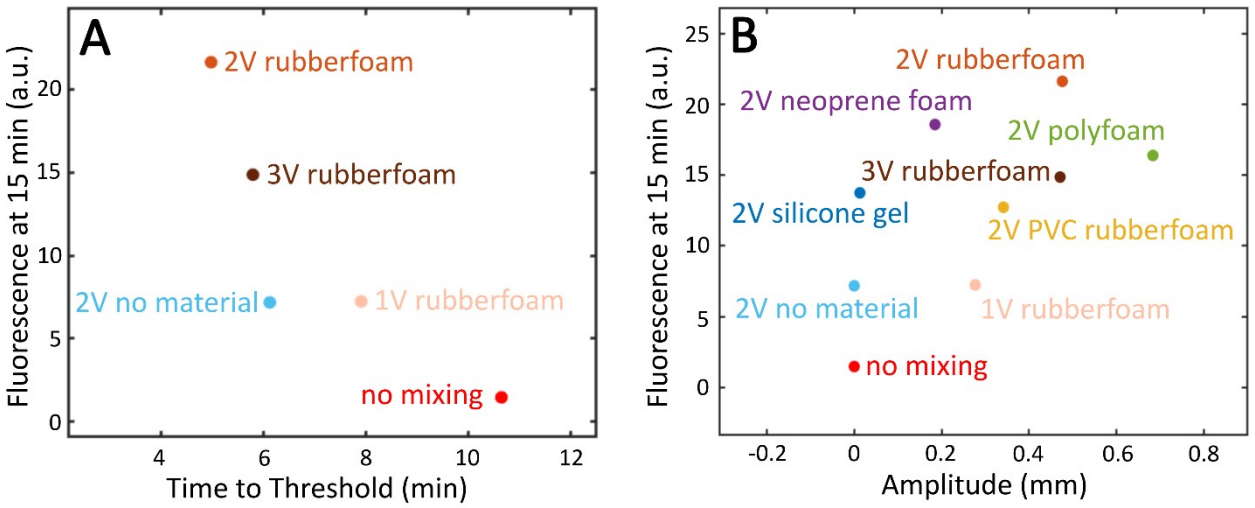

Figure S5. (A) Graph of endpoint fluorescence vs time to threshold for varying motor inputs. (B) Graph of endpoint fluorescence vs Z-amplitude for all materials and motor input voltages tested.

Figure S5A shows the RPA reaction output for the best performing isolation material at varying motor input voltages. While higher motor inputs should increase mixing performance, the high voltage may reduce RPA performance due to various issues such as difficulty in maintaining RPA temperature conditions and possible reduced motor surface contact to the RPA membrane from increased vibrations. Figure S5B shows the RPA reaction for all the isolation materials and motor inputs tested. These datapoints are plotted with the endpoint fluorescence versus z-amplitude measurements. There is a loose correlation between materials with a high z-amplitude having a higher endpoint RPA output.

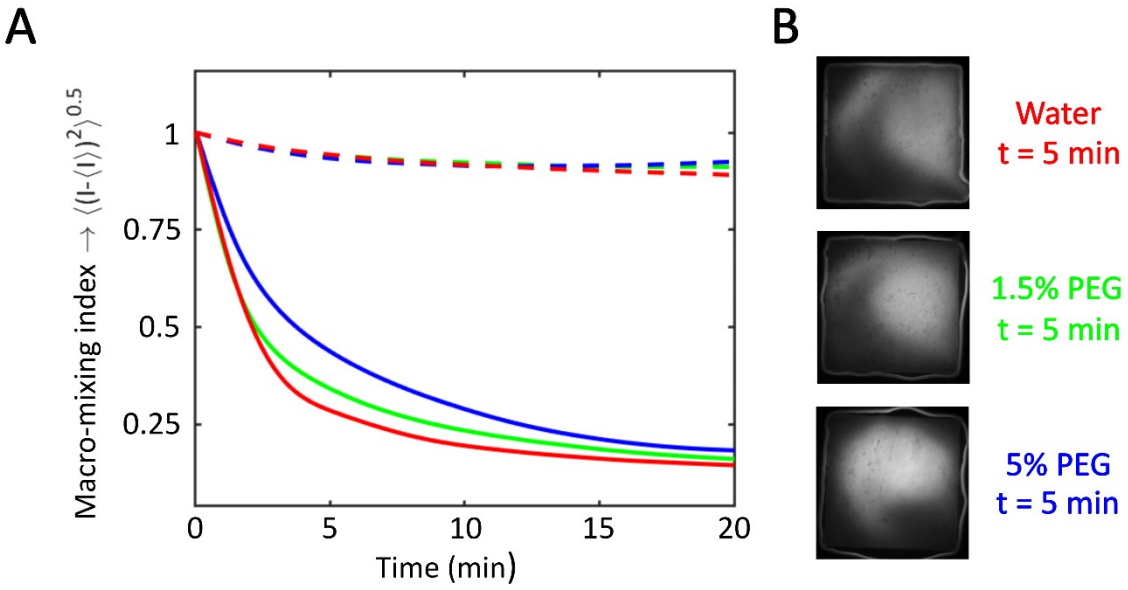

Figure S6. (A) Macro-mixing index vs time for varying PEG concentrations. Dotted lines represent unmixed experiments and Solid lines represent mixed experiments. Red line represent water only in solution, green line represents 1.5% PEG solution, and blue line represents 5% PEG solution. Lines are averaged plots from n=3 experiments. (B) Images of mixed experiments at the 5 minute mark.

Figure S6 shows bulk stirring with varying percentages of PEG in the solution. To perform these experiments, we added 45.5 µL of solution (nuclease-free water with varying PEG percentages) to the GF/DVA membrane. We then pipetted 4.5 µL of fluorescently labeled DNA to the center of the membrane before sealing it with PCR film and inserting it into the platform. We found that for varying percentages of PEG, the platform can effectively stir the labeled DNA. At five minutes, the water only solution reached a macro-mixing index of .28, where 1 represents a fully unmixed experiment. For 1.5% PEG, the macro-mixing index reached 0.34 at 5 minutes. In comparison, the RPA stirring experiments done, in Figure 2, reached a similar mixing index of 0.35. Lastly for 5% PEG, the experiment dropped from an index of 1 to .44 at the end of

5 minutes. Overall, PEG (a highly viscous crowding agent) slightly affects the mixing performance of the platform. Nevertheless, the platform is still able to sufficiently stir high viscosity solutions.

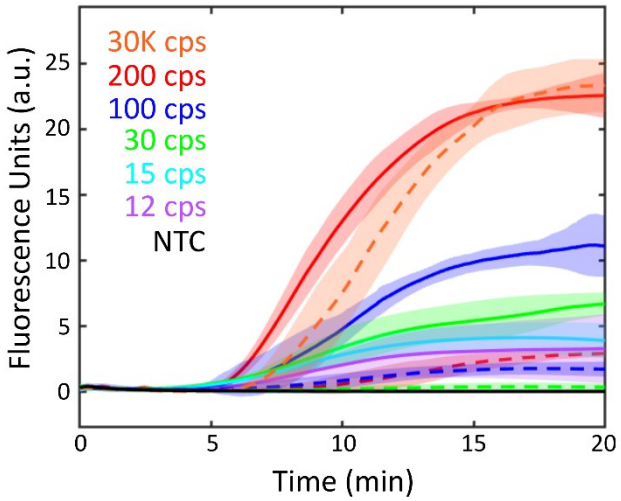

Figure S7. Data from Figure 4B plotted as averages with corresponding standard deviations (shaded regions). Bulk fluorescence as a function of time for unmixed (dashed lines) and mixed (solid lines) reactions. Color indicates specific copy number. Black lines represent the no template controls (NTC) for both mixed and unmixed configurations. For unmixed experiments, 30-30,000 copies per reaction can be seen from (dashed green to dashed orange respectively). For mixed reactions, 12-200 copies per reaction is seen from (solid purple line to solid red line respectively).

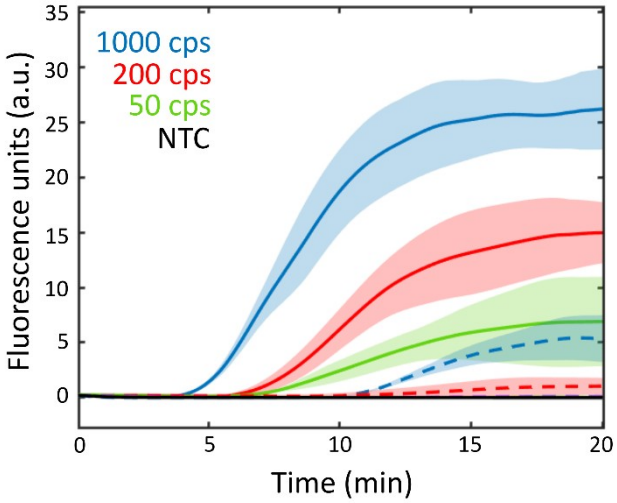

Figure S8. Data from Figure 5 plotted as averages with corresponding standard deviations. HIV RNA results for 1000, 200, and 50 copies per reaction. Solid lines represent mixed reactions and dashed lines represent unmixed reactions. Black lines represent no template controls (NTC).

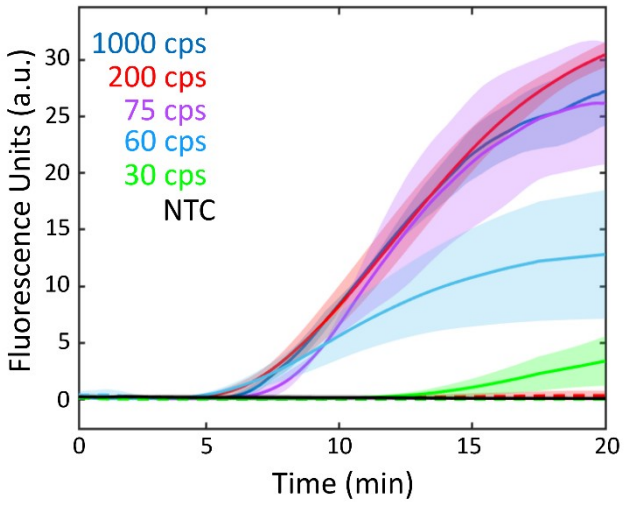

Figure S9. Data from Figure 6B plotted as averages with corresponding standard deviations. Bulk fluorescence results for unmixed reactions (dashed lines) and mixed reactions (solid lines). Color indicates specific copy number. Black lines represent the no template controls (NTC) for mixed and unmixed reactions.

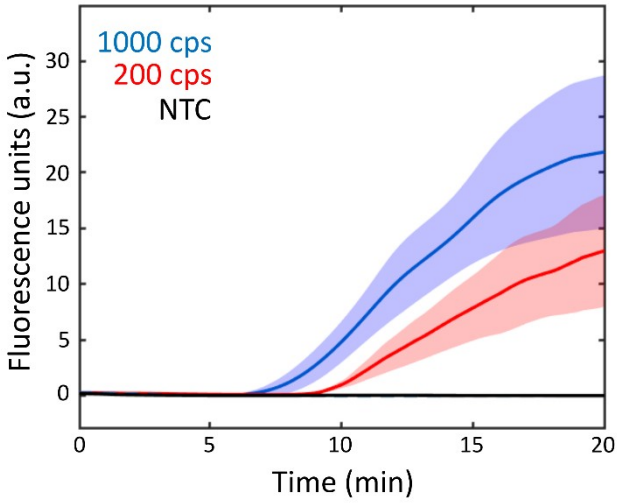

Figure S10. Data from Figure 7 plotted as averages with corresponding standard deviations. Bulk fluorescence plots for 1000 and 200 copies HIV DNA for vibration-based mixing with no PTC. Solid lines represent mixed experiments, dashed lines represent unmixed experiments, and black lines represent no template controls (NTC).

Bulk stirring and other analysis code can be found at DOI: 10.5281/zenodo.12729647 .