

Lack of correlation between reaction speed and analytical sensitivity in isothermal amplification reveals the value of digital methods for optimization: validation using digital real-time RT-LAMP

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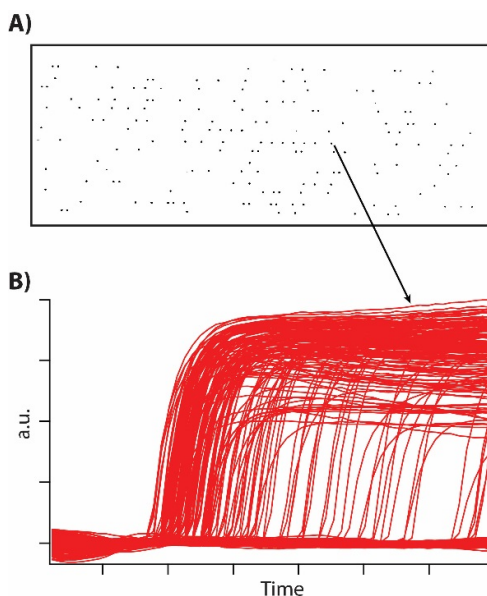
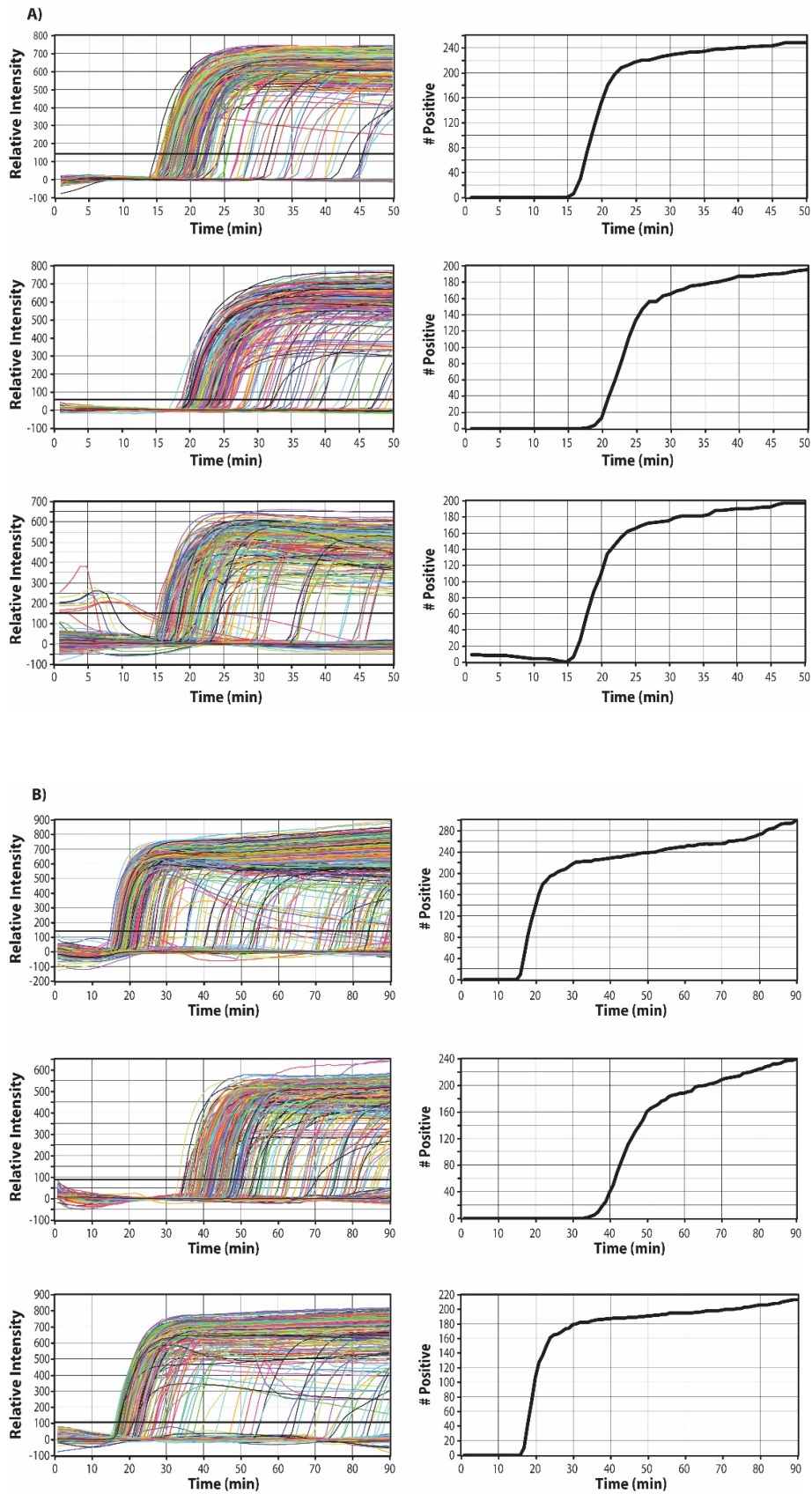


Figure S1. Real time imaging of single template amplification reactions using a 1,280-well SlipChip device. (A) A schematic of the final digital readout at the end point and (B) a plot of single template molecule amplifications in each well, tracked in real time. The arrow indicates the change in intensity (a.u.) over time of a single well due to the amplification process (B) and the same well's location in the device shown in (A).



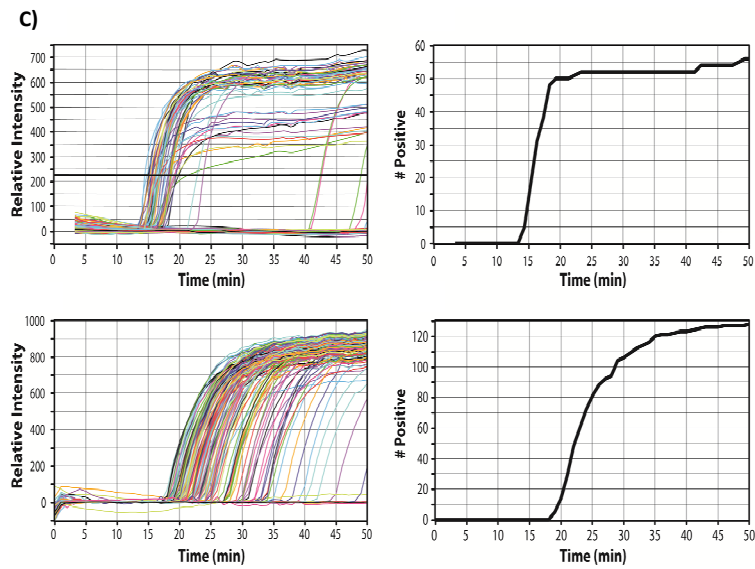


Figure S3. Examples of the data on real-time digital measurements of the cumulative fates and rates of the single template molecules. (A) when comparing the digitally optimized primer set (DOP) (top) and elongated BIP and FIP sets (DOP-LBIP and DOP-LFIP) (middle and bottom); (B) when comparing the digitally optimized primers sets with both loop primers (DOP) (top), with no loop primers (DOP-NL) (middle) and with no loop F primer only (DOP-NLF) (bottom); (C) when comparing two different enzymes mixtures, EM (top) and RtxBst (bottom).