

Supplementary Information

Palm-size and one-inch gel electrophoretic device for reliable and field-applicable analysis of recombinase polymerase amplification

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Supplementary Figures:

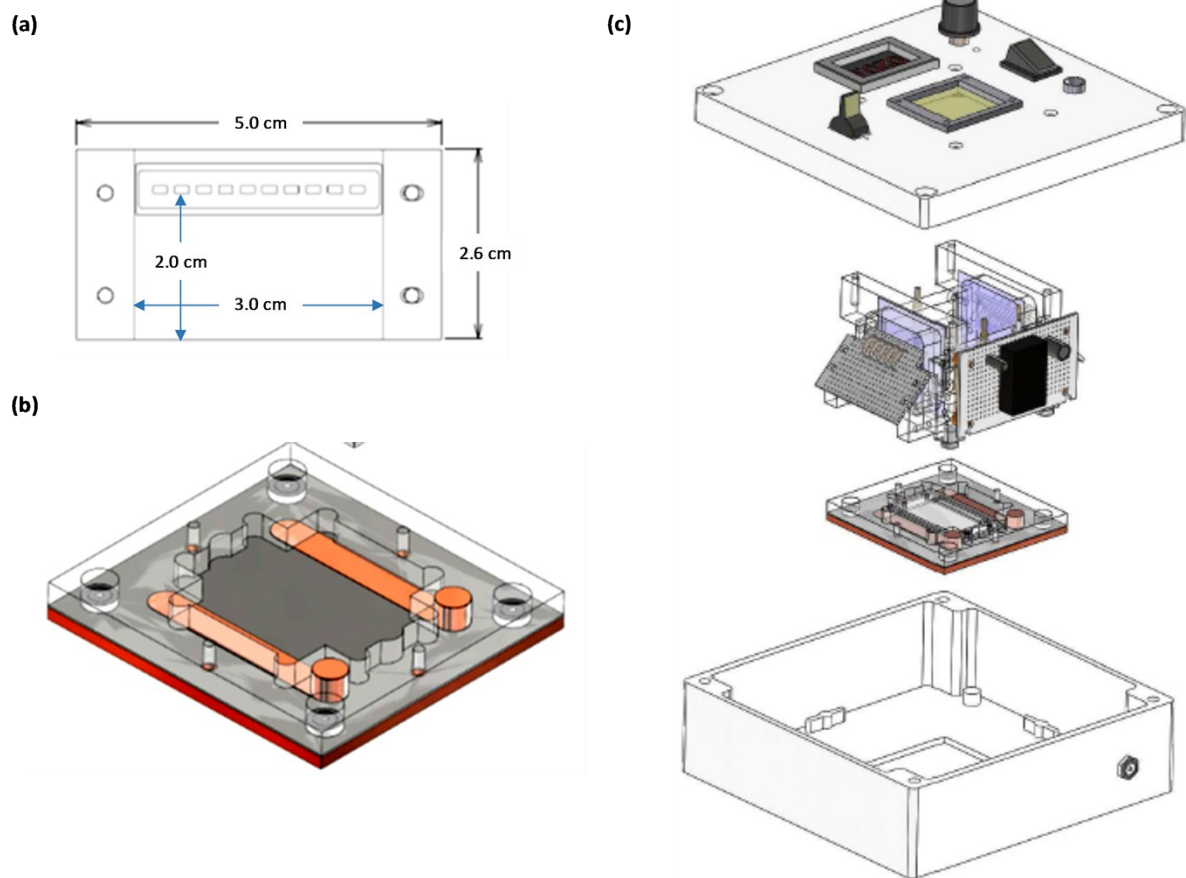


Figure S1: Sectional view of hand-held gel electrophoretic device. (a) Gel cassette used to prepare pre-cast gel for on-site electrophoresis (b) Gel cassette holder to perform horizontal gel electrophoresis (c) the complete system including power supply, horizontal gel electrophoresis platform, and gel imaging system which provides a viable solution for on-site documenting and analysing polyacrylamide gel electrophoresis-based results.

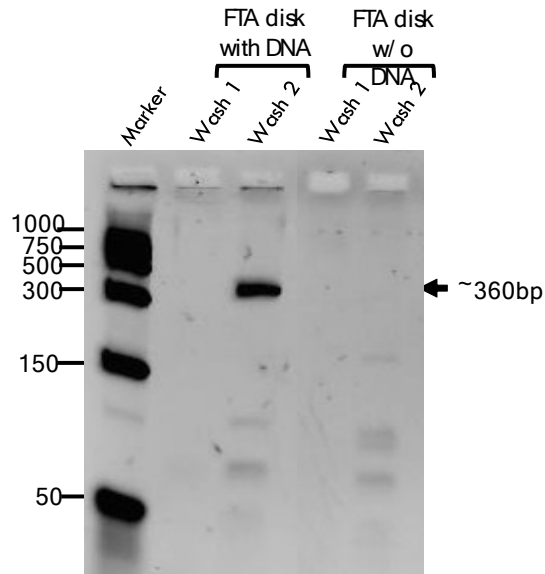


Figure S2: Evaluation of FTA card for RPA. *L.major* 5ASKH culture was spotted onto the FTA card and 2.0-mm-diameter discs were punched. All the discs were washed with 50 μ l FTA purification reagent followed by another wash using 500 μ l of TE buffer. The washed FTA card discs were directly used for amplification using RPA for 15 minutes. Amplification was successful only in the case of Wash 2 which indicates inhibition of RPA due to incomplete removal of FTA purification reagents which is the case of Wash 1. (Wash 1: Washing with FTA wash buffer (1X); Wash 2: Washing with FTA wash buffer (1X) and TE buffer (1X)).

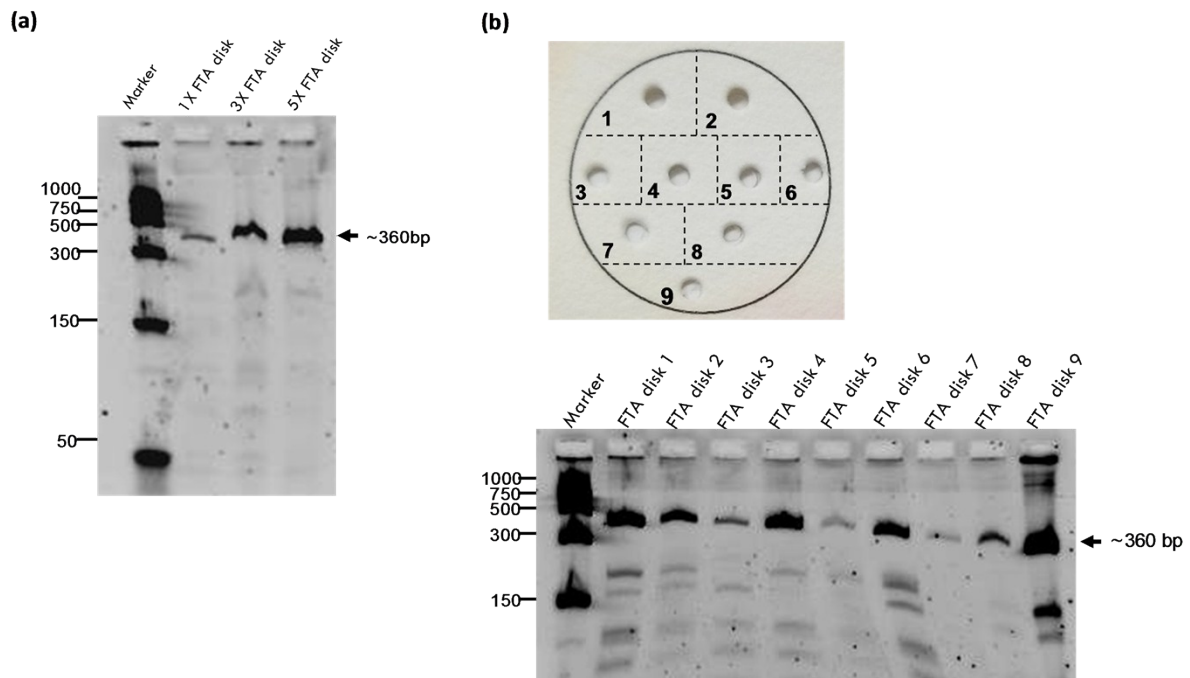


Figure S3: Evaluation of FTA card for RPA (cont.). *L.major* 5ASKH culture was spotted onto the FTA card and 2.0-mm-diameter discs were punched at 9 different positions from areas as shown in (b). All the disks were washed with 50 μ l FTA purification reagent, then 2 times with 500 μ l TE buffer. Then, the FTA card disks were directly used for amplification using RPA for 15 minutes. Amplification efficiency was dependent upon the concentration of DNA present in the FTA disk. The order of intensity of RPA products obtained from the position of disk is: #9 > #1, 4, 6 > #2 > #3, 8 > #5, 7 which confirms that DNA is not distributed uniformly on the FTA card. Therefore, multiple disks are advices to increase product yield in RPA (a). Wash 1: Wash with FTA wash buffer (1X); Wash 2: Wash with FTA wash buffer (1X) and TE buffer (2X).

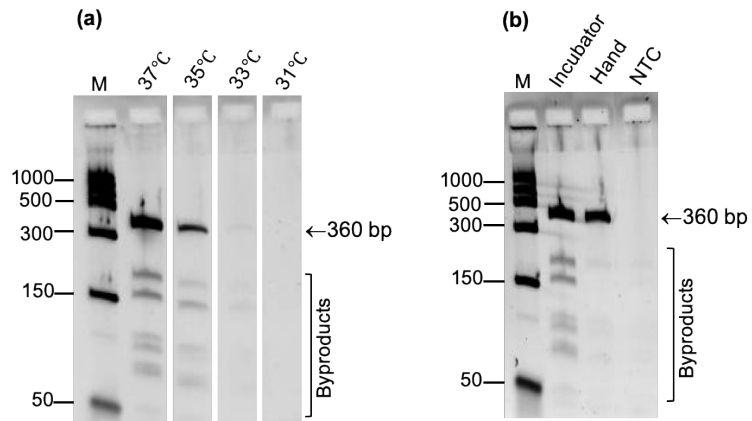


Figure S4: RPA was performed using FTA card with 0.24 μ M each primer and 15 min incubation time targeting 18S rRNA gene segment (a) at temperatures ranging from 37°C-31°C using incubator and (b) at 34°C using incubator (lane 2) and by holding in hand (lane 3). 1 ul of each product was electrophoresed and analyzed using UV transilluminator. (NTC: No-template control)

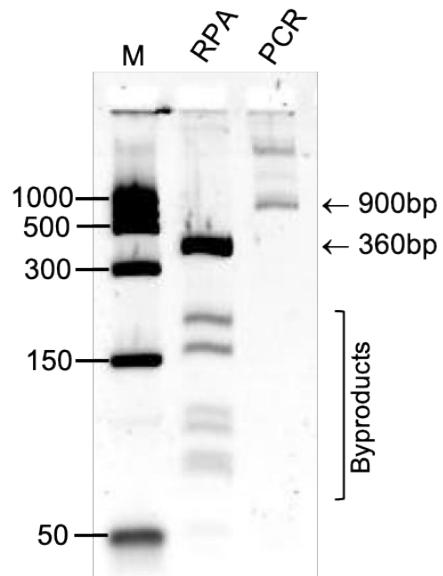


Figure S5: FTA disks were used as template, RPA (Lane 2) was performed using RPA primers targeting 18S rRNA gene segment of 360 bp and PCR (Lane 3) was performed using PCR primers targeting minicircle gene segment of 900 bp. The products were analyzed using electrophoresis.