Supporting information

Development and Evaluations of RPA-NFO-LFT and RPA-Cas12a-LFT Systems for the Detection of *Candida albicans*

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Fig. S1

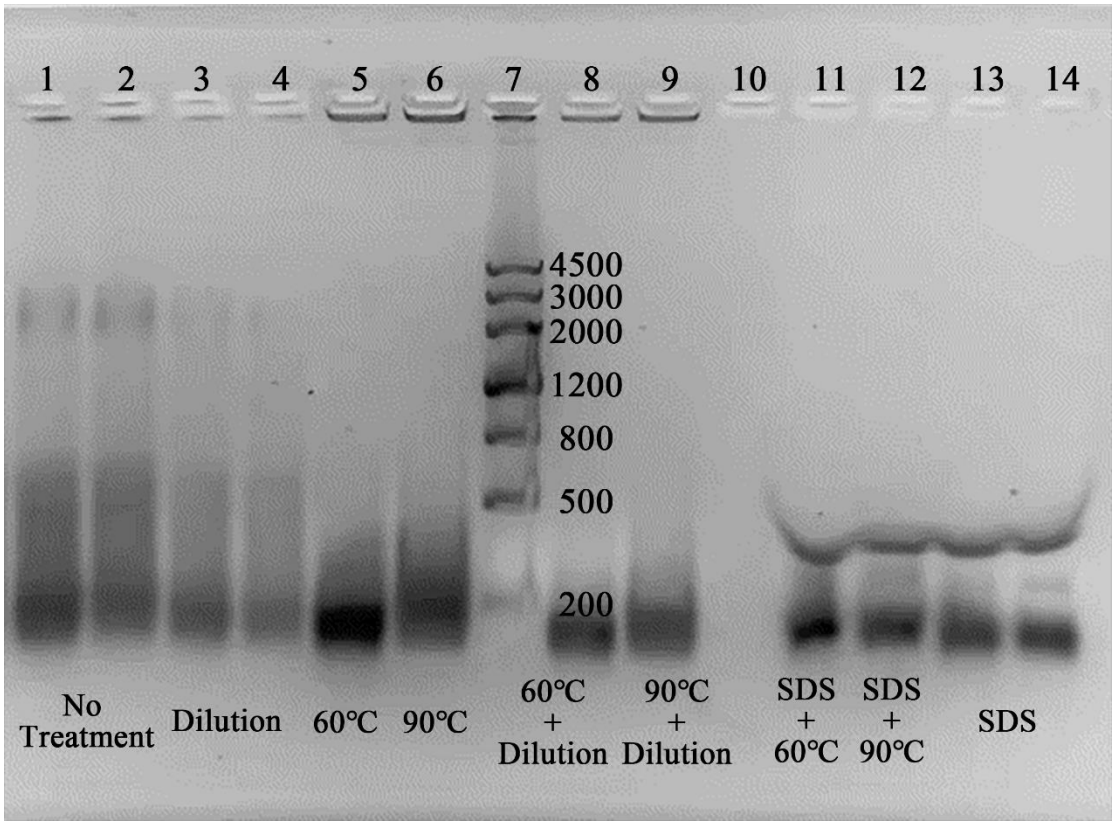

Fig. S1 RPA product analysis after different purification treatments

As can be seen from the lane 1 and lane 2, the bands are heavily smeared at 3000 bp, indicating the appearance of a DNA-protein complex. Lane 3 and lane 4 reflected the dilution has few effects on the separation of DNA and protein. High temperature treatment is beneficial for the separation of amplicons from enzymes and proteins. The bands from the 90 °C treatment (lane 6) are significantly less concentrated than those from the 60 °C treatment (lane 5), because high temperature denatures the amplicons into single strands that cannot be detected by electrophoresis. In lanes 8 and 9, high temperature denaturation followed by dilution obviously restrains the smearing, but also leads to the reducing of RPA product. The SDS treated samples were shown in Lane 11 to 14. Although it is the most effective in eliminating the smearing, the interference band above the target is inevitable, especially when the amplicon is long. Therefore, the 60 °C treatment was finally chosen as the post-processing method for the characterization of RPA product.

Fig. S2

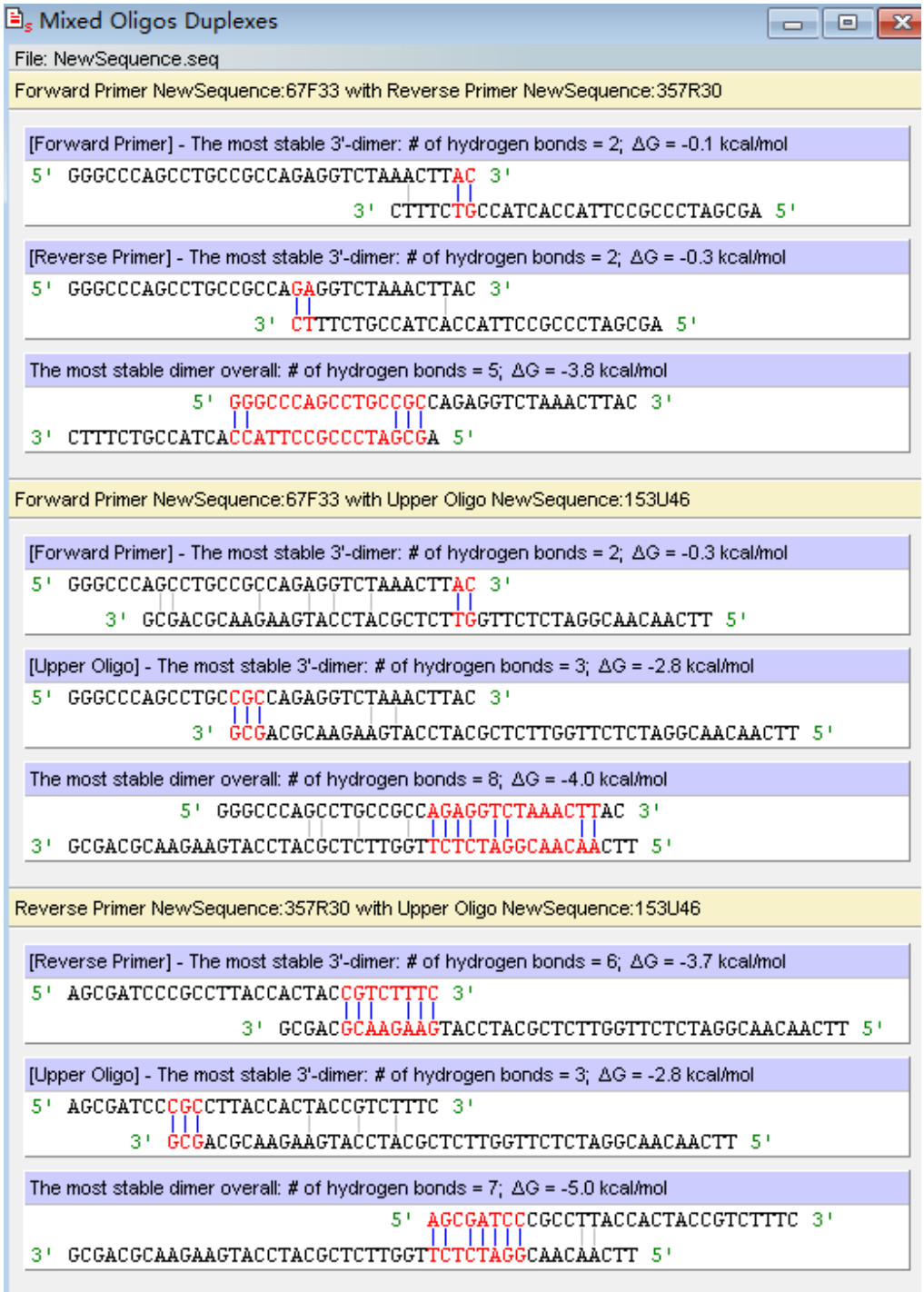

Fig. S2 Results of secondary structure energy calculations for primers and probes

Fig. S3

Fig. S3 Bare test strips and finished test strips after shelling.

Fig. S4

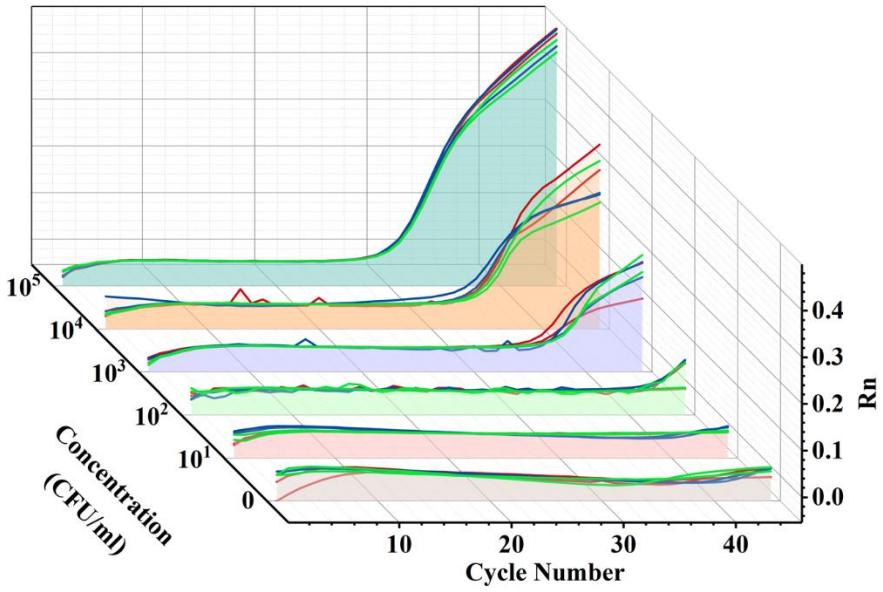

Fig. S4

Amplification curves of qPCR assay for interference samples, curves of the same color at the same concentration represent duplicate experiments, and curves of different colors represent parallel samples. The six curves were highly overlapping at a concentration of 10^5 CFU/ml and the assay was very stable, with increasing fluctuations in fluorescence values as the sample concentration decreased. Lower limit of detection up to 10^3 CFU/ml in 45 cycles, and the trend of the curve shows that increasing the number of cycles should allow the detection of lower concentrations. Theoretically, the CT value detected by qPCR should increase by 3.16 when the sample concentration decreases by a factor of 10. However, the mean CT value of samples tested at 10^4 CFU/ml increased by 3.76 compared to that of 10^5 CFU/ml under the influence of serum background. When testing samples with 10^3 CFU/ml concentration, one duplicate well showed negative, and after excluding this data, the mean CT value of samples with 10^3 CFU/ml concentration was calculated and found to be increased by 4.42 compared to the mean CT value of 10^4 CFU/ml. Background nucleic acids reduced the amplification efficiency of qPCR to some extent and the lower the concentration of the sample, the greater the effect on the amplification efficiency.

Fig. S5

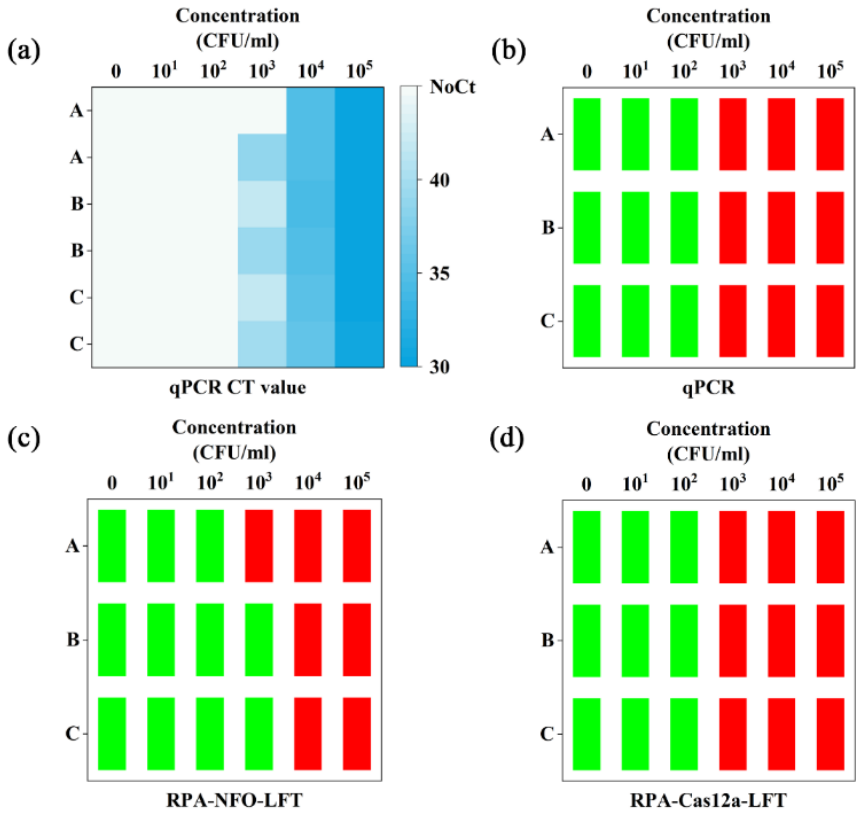

Fig. S5 Results of qPCR and RPA-NFO/Cas12a-LFT on 18 simulation samples. (a) Ct values of qPCR assay for 18 samples, with one replicate experiment for each sample. (b, c, d) Results of qPCR, RPA-NFO-LFT, and RPA-Cas12a-LFT on 18 samples tested for negative and positive readings, with green as negative and red as positive.

Table S1 Mismatch screening primer sequence

Name	Forward Primer (5'->3')	Reverse Primer (5'->3')
ITS1-2-3	GGGCCCAGCCTGCCGCCAGAGGTCTAAACTTAC	CAAAGCGATCCCGCCTTACCACTACCCGTCT
ITS1-2-2	GGCCCAGCCTGCCGCCAGAGGTCTAAACTTACA	AAAGCGATCCCGCCTTACCACTACCGTCTT
ITS1-2-1	GCCCAGCCTGCCGCCAGAGGTCTAAACTTACAA	AAGCGATCCCGCCTTACCACTACCGTCTTT
ITS1-2+1	CCAGCCTGCCGCCAGAGGTCTAAACTTACAACC	GCGATCCCGCCTTACCACTACCGTCTTTCA
ITS1-2+2	CAGCCTGCCGCCAGAGGTCTAAACTTACAACCA	CGATCCCGCCTTACCACTACCGTCTTTCAA
ITS1-2+3	AGCCTGCCGCCAGAGGTCTAAACTTACAACCAA	GATCCCGCCTTACCACTACCGTCTTTCAAG

Table S2 qPCR procedure, primer and probe sequence

Step	Temperature (℃)	Data collection	Time	Number of cycles	Analysis Mode
Pre-denaturation	95	None	2 min	1	None
Extension	95	None	10 sec	45	Quantification
	60	Single	40 sec		
Cooling	37	None	10 sec	1	None

Name	Sequence (5'->3')
Forward Primer	GGATCTCTTGGTTCTCGCATC
Reverse Primer	GTAGTCCTACCTGATTTGAGGTC
Probe	HEX-GCCATTGTCAAAGCGATC-BHQ1

PCR reactions included 0.5 μl (10 μM) of forward primer, reverse primer and probe, 5 μl of AptaTaq Genotyping Master (Roche), 5 μl of template, and 13.5 μl of water.