

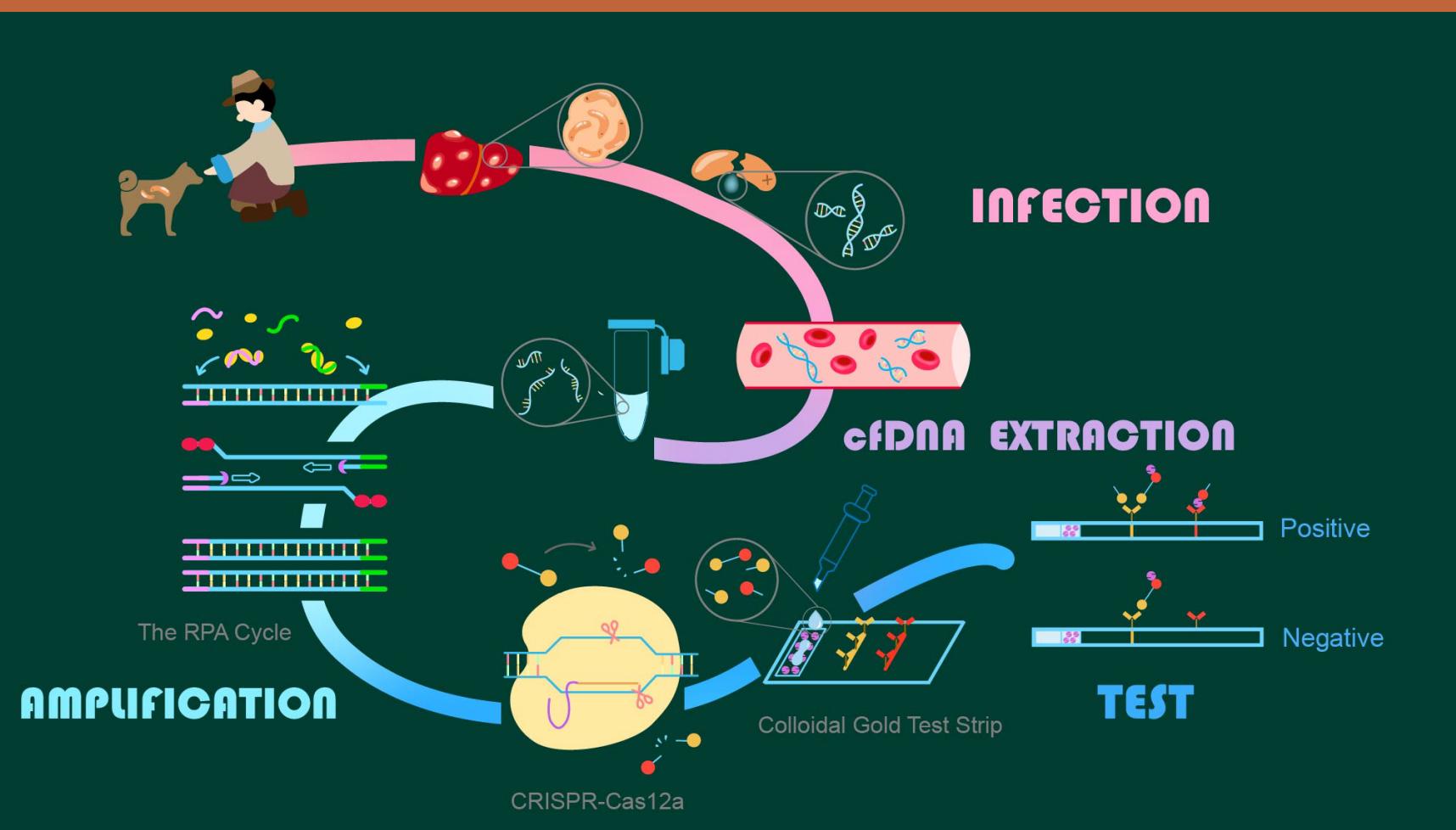
# WANTED

## Diagnosing echinococcosis through multi-sgRNA bulletin of circuit free DNA using CRISPR-Cas12a



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Our diagnostic system for parasitic diseases tests the person's circulating cell-free DNA (cfDNA) in their blood to see if they are infected with the parasite. We used the Recombinase Polymerase Amplification (RPA) to amplify cfDNA in blood. Then the amplification product was used to activate the trans-cleavage activities of CRISPR-Cas12a system, cut the nucleic acid probe we designed. Finally, the colloidal gold test stripes were used to detect the cleavage product.

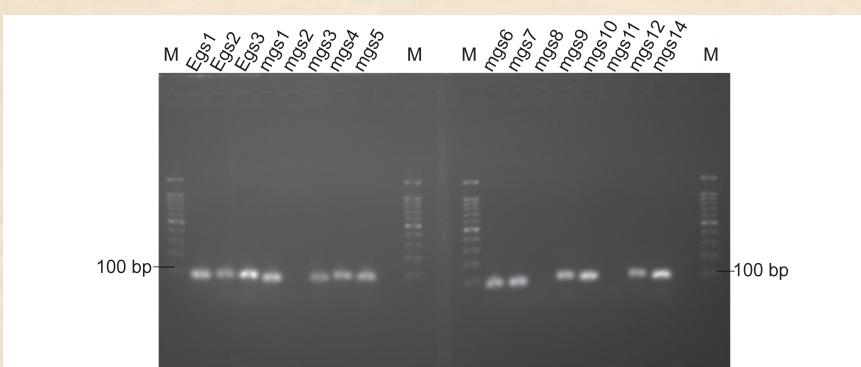


Figure 1. Amplification of the target fragment. Target fragments were amplified by PCR using 16 pairs of cfDNA predictive primers, the amplified products were subjected to 2% agarose electrophoresis at 140 V for half an hour.

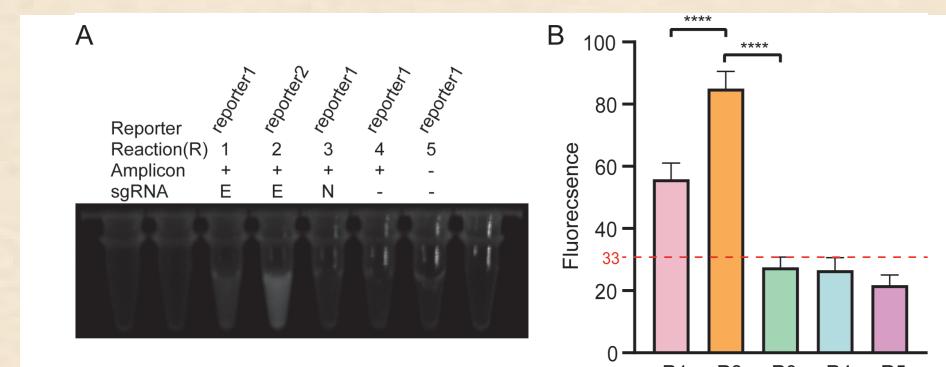


Figure 3. CRISPR-Cas12a detects amplified target fragments. (A) The fluorescence of reporters was detected under UV light. The concentration of reporter 1(8 nt) or reporter 2(15 nt) is 500 nM; Amplicon, The amplified E gene fragment; E, the sgRNA of SARS-CoV-2 E gene; N, the sgRNA of SARS-CoV-2 N gene. (B) Fluorescence reader detects different reaction. The red dashed line indicates the Max fluorescence intensity of reaction 3. \*\*\*\*, p < 0.0001.

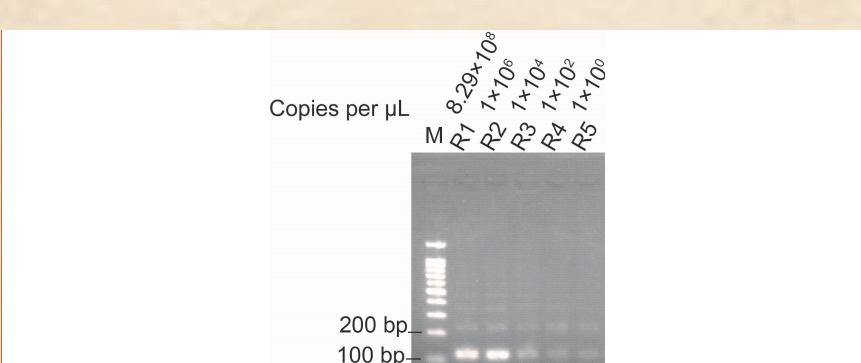


Figure 2. Recombinase polymerase amplifies the target fragment. Recombinase polymerase amplification (RPA) was performed using templates with different copy numbers (R1-R5). Then purifying the amplification products with phenol-chloroform method and using agarose electrophoresis to test the purified DNA.

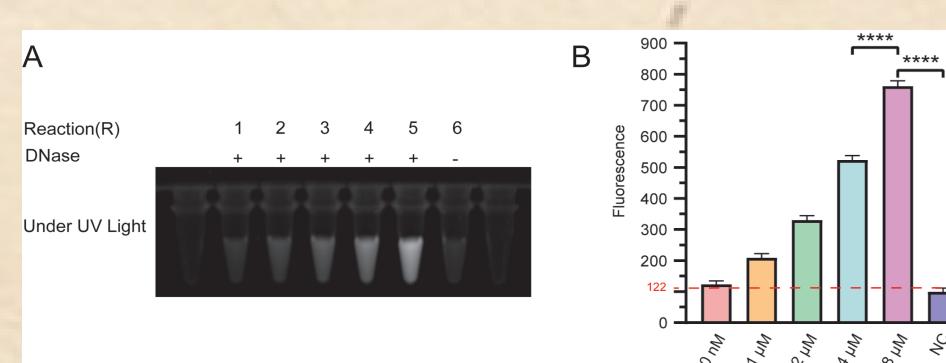


Figure 4. DNase digests fluorescent reporter (15 nt). (A) Reporter fluorescence under UV light. Reporter concentration: R1, 500 nM; R2, 1  $\mu$  M; R3, 2  $\mu$  M; R4, 4  $\mu$  M; R5, 8  $\mu$  M; R6(NC), 500 nM, without DNase. (B) Fluorescence intensity detected by fluorescence reader. The red dashed line indicates the Max fluorescence intensity of the NC. \*\*\*\*, p < 0.0001.

## CONCLUSION

- We selected several primers with better amplification efficiency among 16 pairs of cfDNA predictive primers by PCR.
- We successfully amplified the target fragment using RPA at a low copy number (1 copy).
- We used the Target fragment amplified by RPA to activate the trans-cleavage activities of CRISPR-Cas12a system, cut the fluorescent reporter we designed and synthesized.

We try to extract cfDNA from human blood, then detect the cfDNA using our system to diagnose parasitic diseases including the final colloidal gold test papers detection. In addition, we try to optimize CRISPR-Cas12a nucleic acid detection system by multi-sgRNA.

**RESULTS**