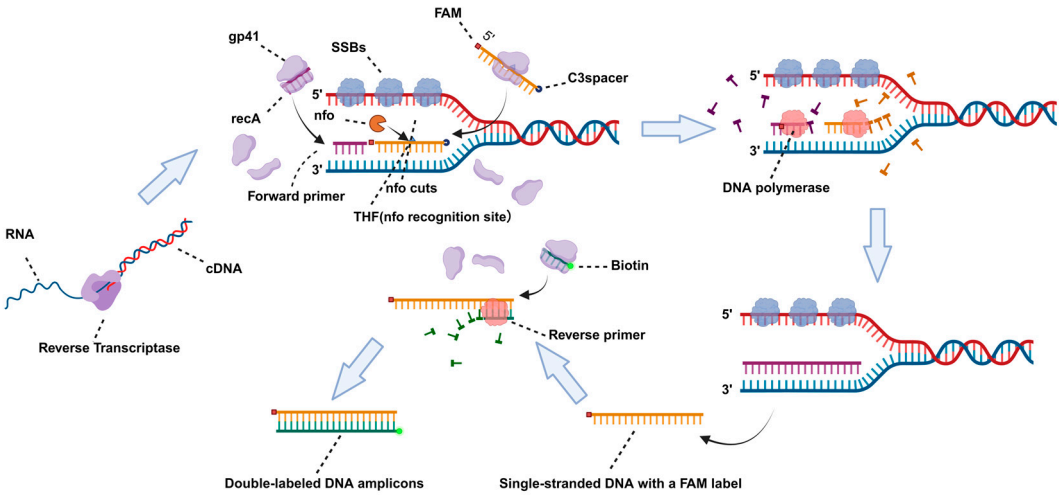
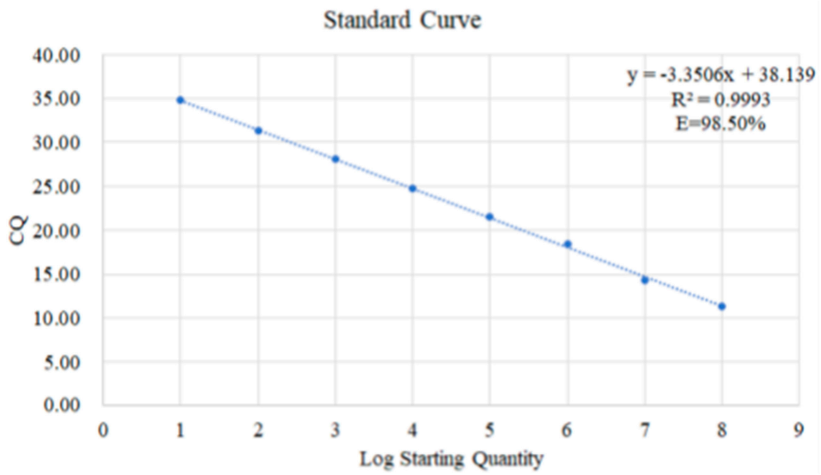
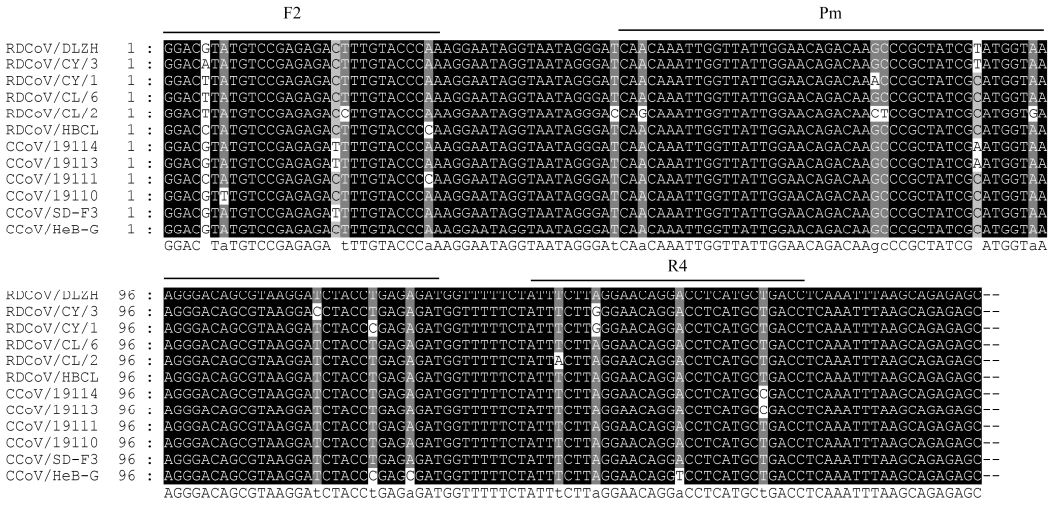


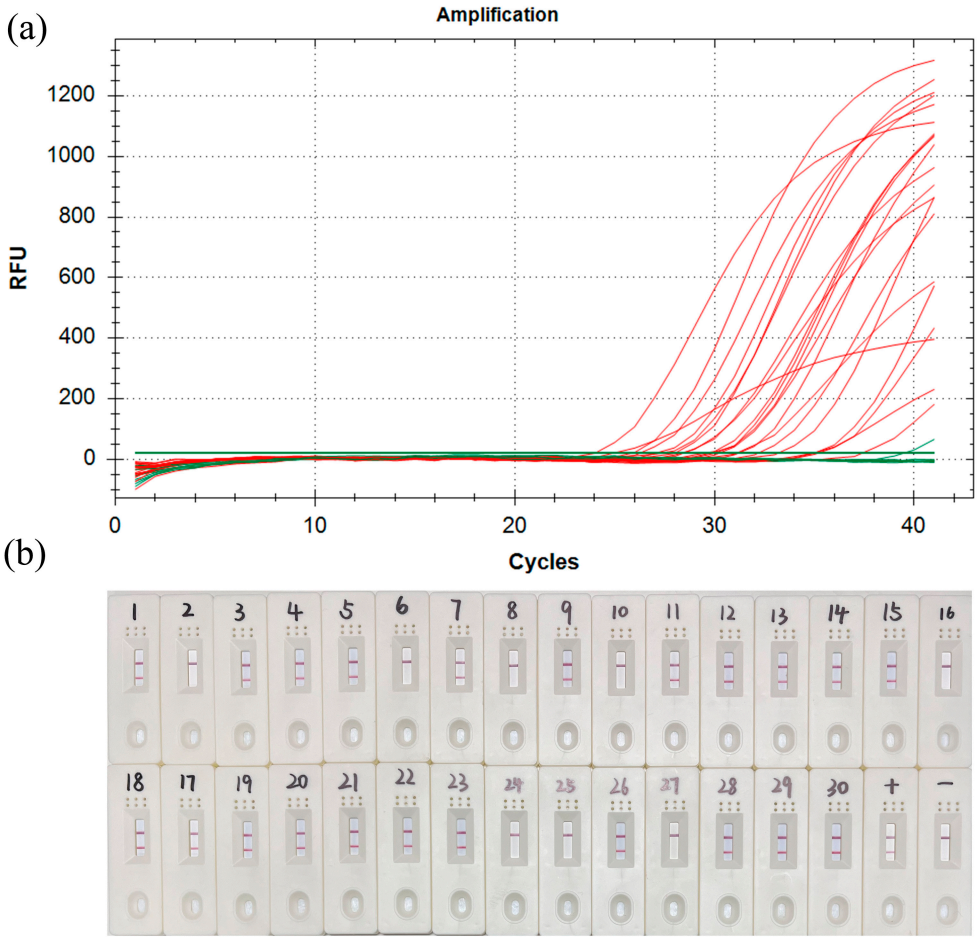
Supplementary Figure S1. Schematic illustration of the one-step RT-MIRA amplification mechanism. Reverse transcriptase first converts the viral RNA template into cDNA. The MIRA reaction utilizes a novel recombinase (helicase gp41 and recA) and single-stranded binding proteins (SSBs) to facilitate primer invasion into the double-stranded DNA. The reverse primer is 5'-biotinylated, while the probe contains a 5'-FAM label and a THF (tetrahydrofuran) residue recognized by endonuclease IV (nfo). Upon nfo cleavage, DNA polymerase extends the primers, generating double-labeled DNA amplicons (FAM and biotin) and single-stranded DNA with a FAM label. This figure was created with BioRender.com.



Supplementary Figure S2. Standard curve of the RT-qPCR assay. The standard curve was generated using 10-fold serial dilutions of the recombinant pMD-20T-N plasmid. The x-axis represents the logarithm of the starting quantity (Log Starting Quantity), and the y-axis represents the threshold cycle (Cq). The regression equation is y=−3.3506x+38.139, with a correlation coefficient (R^2) of 0.9993, and an amplification efficiency of 98.50%.



Supplementary Figure S3. The position of probe and primers of MIRA-LFD on the RDCoV/CL/2022 genome.



Supplementary Figure S4. Detection results of the 30 clinical samples.**(a)** Amplification curves of the RT-qPCR assay. The red curves represent the positive samples, while the green flat lines represent the negative samples. **(b)** Visual readout of the MIRA-LFD assay. The 30 clinical samples are numbered 1–30. Positive samples show two red lines (Control line and Test line), and negative samples show only one red line (Control line). "+" indicates the positive control, and "−" indicates the negative control.

Supplementary Table S1. Specimen-level details and clinical evaluation results of the 30 raccoon dog fecal samples.

Sample ID	Farm Origin	Collection Date	Clinical Status	RT-qPCR Ct Value	RT-qPCR Result	MIRA-LFD Result
1	Jiling city	2022/11/29	Diarrhea	27.26	Positive	Positive
2	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
3	Jiling city	2022/11/29	Diarrhea	28.41	Positive	Positive
4	Jiling city	2022/11/29	Diarrhea	24.00	Positive	Positive
5	Jiling city	2022/11/29	Diarrhea	26.24	Positive	Positive
6	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
7	Jiling city	2022/11/29	Diarrhea	29.73	Positive	Positive
8	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
9	Jiling city	2022/11/29	Diarrhea	32.16	Positive	Positive
10	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
11	Jiling city	2022/11/29	Diarrhea	29.45	Positive	Positive
12	Jiling city	2022/11/29	Diarrhea	29.98	Positive	Positive
13	Jiling city	2022/11/29	Asymptomatic	37.44	Positive	Positive
14	Jiling city	2022/11/29	Diarrhea	28.29	Positive	Positive
15	Jiling city	2022/11/29	Diarrhea	24.88	Positive	Positive
16	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
17	Jiling city	2022/11/29	Diarrhea	33.16	Positive	Positive
18	Jiling city	2022/11/29	Diarrhea	27.02	Positive	Positive
19	Jiling city	2022/11/29	Diarrhea	33.96	Positive	Positive
20	Jiling city	2022/11/29	Diarrhea	35.15	Positive	Positive
21	Jiling city	2022/11/29	Diarrhea	31.09	Positive	Positive
22	Jiling city	2022/11/29	Diarrhea	34.88	Positive	Positive
23	Jiling city	2022/11/29	Diarrhea	28.01	Positive	Positive
24	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
25	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
26	Jiling city	2022/11/29	Diarrhea	25.81	Positive	Positive
27	Jiling city	2022/11/29	Asymptomatic	N/A	Negative	Negative
28	Jiling city	2022/11/29	Diarrhea	31.17	Positive	Positive
29	Jiling city	2022/11/29	Diarrhea	30.21	Positive	Positive
30	Jiling city	2022/11/29	Asymptomatic	37.37	Positive	Positive