Supporting Information

Sensitive and Robust One-Pot RPA-CRISPR/Cas12a Assay with Elimination of *Cis*-Cleavage

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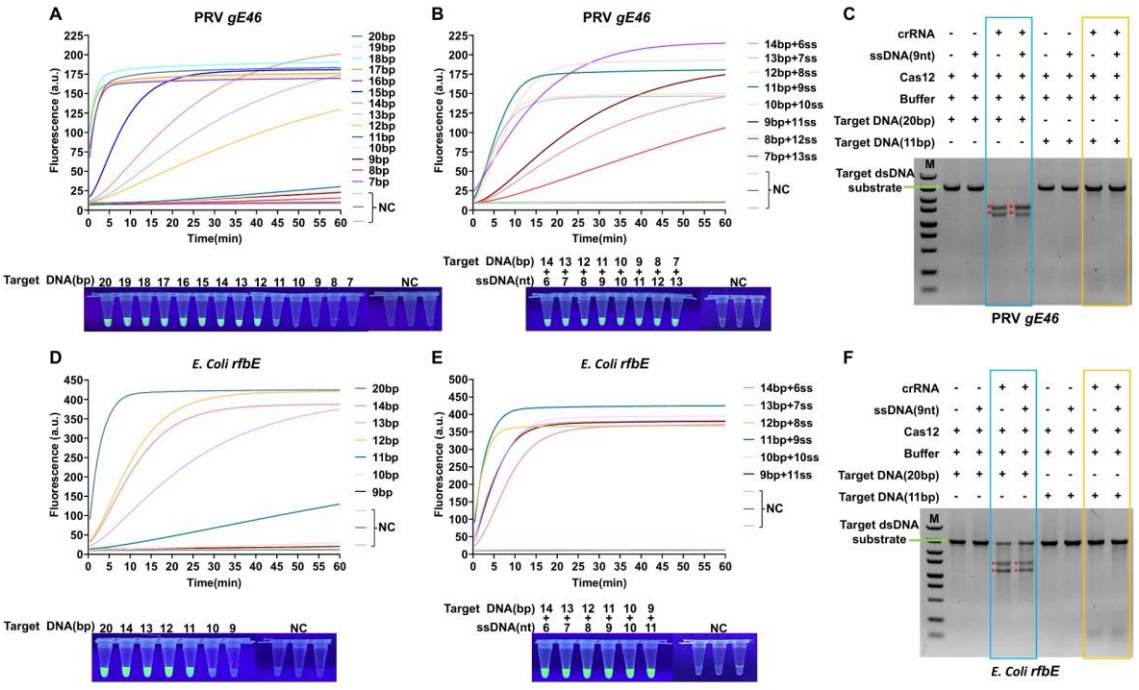


Figure S1. Other genes used to verify the *cis*- and *trans*-cleavage activities of LbCas12a on target dsDNA and target ssDNA of different lengths.

(A, D) 45bp hairpins containing target dsDNA of varying lengths were designed for crRNA targeting PRV *gE46* and *E. Coli rfbE*, to evaluate the activation effect on *trans*-cleavage activity and the results of direct observation under blue light. (B, E) Design of ssDNA for crRNAs targeting PRV *gE46* and *E. Coli rfbE*, as well as 45bp hairpins with target dsDNAs of different lengths, to evaluate the activation effect on *trans*-cleavage activity and the direct observation results under blue light. (C, F) The 11bp+9ss strategy was used for crRNAs targeting PRV *gE46* and *E. Coli rfbE*, and the *cis*-cleavage activity of this scheme was validated through in vitro cleavage assays. The green arrow indicates the DNA substrate with a total length of approximately 3000bp, containing an 11bp target sequence. The red arrow represents the cleavage fragment. M represents the 5000bp marker. NC represents three negative controls without added DNA substrate, crRNA, and LbCas12a, respectively.

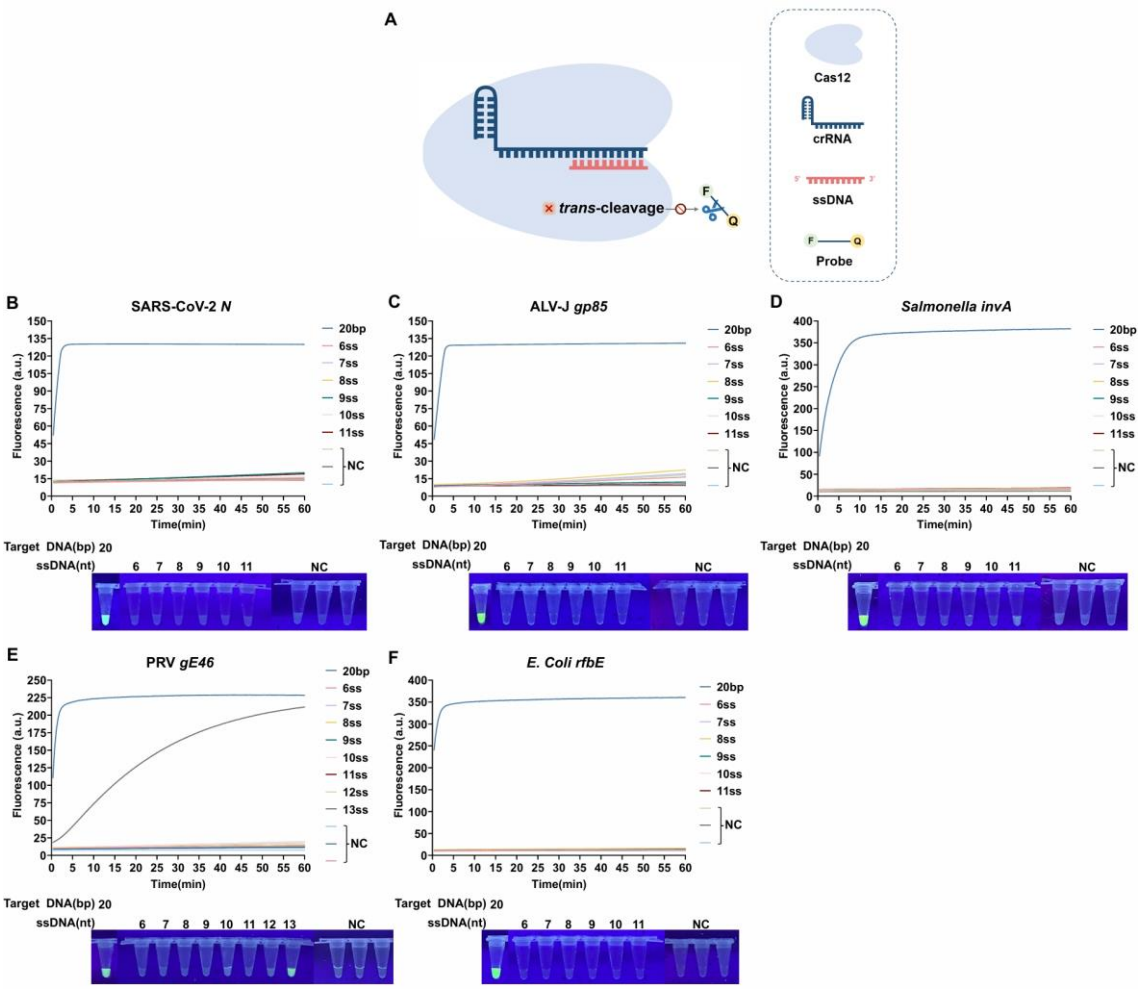


Figure S2. The effect of ssDNA of different lengths on the *trans*-cleavage activity of LbCas12a.

(A) Schematic diagram of the activation of *trans*-cleavage activity of LbCas12a by target ssDNA. (B-F) Design target ssDNA of different lengths based on the crRNAs for SARS-CoV-2 *N*, ALV-J *gp85*, *Salmonella invA*, *E. coli rfbE*, and PRV *gE46*, to evaluate the activation effect on *trans*-cleavage activity and the direct observation results under blue light. NC represents negative controls without DNA substrate, without crRNA, or without LbCas12a, respectively.

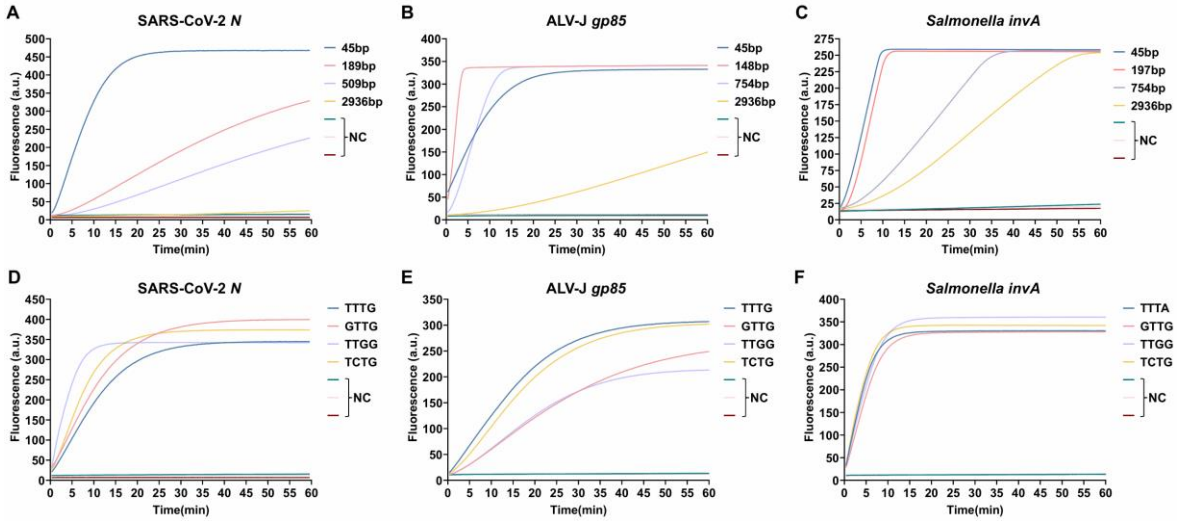


Figure S3. *Trans*-cleavage activity of the 11bp+9ss strategy for DNA substrates of different lengths and different PAMs.

(A-C) Plasmids containing SARS-CoV-2 *N*, ALV-J *gp85*, and *Salmonella invA* sequences were used to amplify four types of DNA substrates with lengths ranging from 45 to 3000 bp, each carrying the 11bp target sequence, for the detection of *trans*-cleavage activity in the 11bp+9ss strategy. (D-F) Hairpins of 45bp carrying an 11bp target sequence and either a conventional PAM (TTTV) or suboptimal PAMs (VTTV/TTVV/TCTV) were designed for SARS-CoV-2 *N*, ALV-J *gp85*, and *Salmonella invA* to assess the *trans*-cleavage activity of the 11bp+9ss strategy. NC represents negative controls without DNA substrate, crRNA, or LbCas12a, respectively.

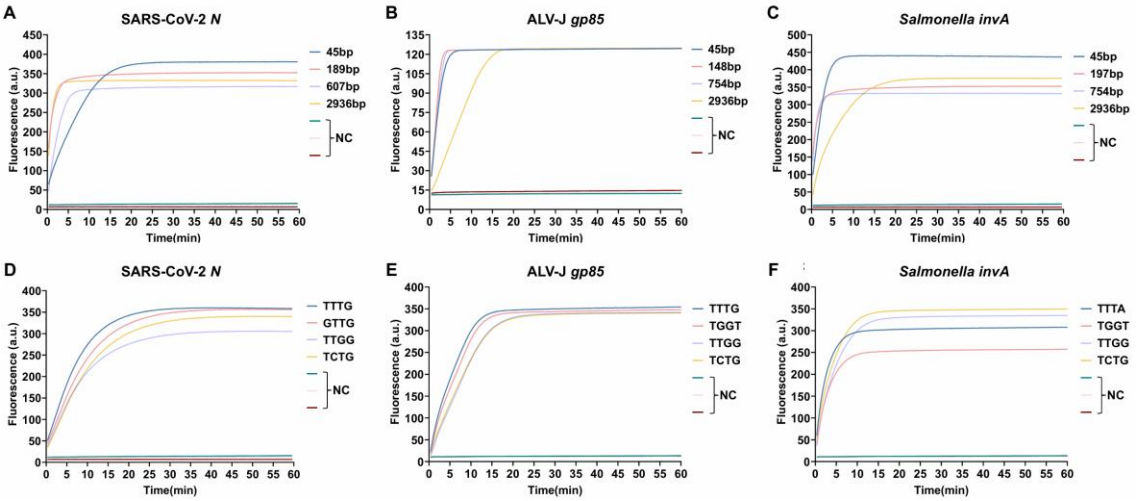


Figure S4. *Trans*-cleavage activity of the conventional 20bp-dsDNA scheme for DNA substrates of different lengths and different PAMs.

(A-C) Four types of DNA substrates, with lengths ranging from 45 to 3000bp, were amplified using plasmids containing SARS-CoV-2 *N*, ALV-J *gp85*, and *Salmonella invA*, respectively. The *trans*-cleavage activity of the conventional 20bp-dsDNA scheme was then assessed for comparison with the 11bp+9ss strategy. (D-F) 45bp hairpins containing 11bp target sequences with either conventional PAM (TTTV) or suboptimal PAMs (VTTV/TTVV/TCTV) were designed for SARS-CoV-2 *N*, ALV-J *gp85*, and *Salmonella invA* to assess the *trans*-cleavage activity of the 20bp dsDNA scheme, serving as a comparison with the 11bp+9ss strategy. NC represents negative controls without DNA substrate, without crRNA, or without LbCas12a, respectively.

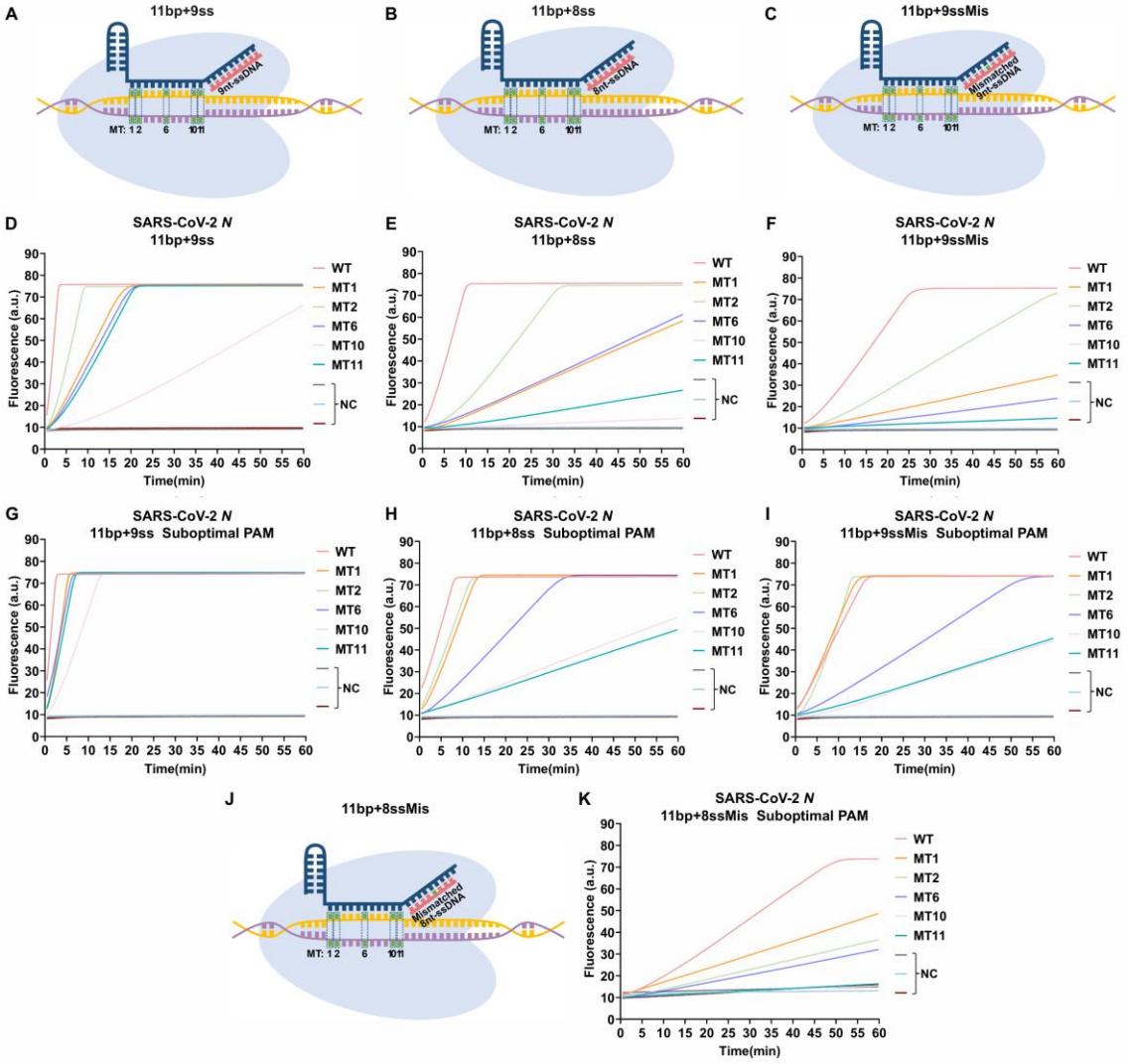


Figure S5. SNV discrimination after optimizing the ssDNA in the 11bp+9ss strategy.

(A-C, J) Schematic diagram of the optimization of the 11bp+9ss strategy. (A) The conventional 11bp+9ss strategy proposed in this study. (B) Shortening the 9nt ssDNA by 1nt, denoted as 11bp+8ss. (C) Introducing 1nt mismatch in the middle of the 9nt ssDNA, with the green square indicating the position of the mismatched base, denoted as 11bp+9ssMis. (J) Shortening the 9nt ssDNA by 1nt while introducing 1nt mismatch at the center, with the green square indicating the position of the mismatched base, denoted as 11bp+8ssMis. (D-I) MT1, 2, 6, 10, and 11 respectively represent five mutations designed at positions 1, 2, 6, 10, and 11 on the target sequence of the 45bp hairpin. Subsequently, the five simulated SNV conditions were identified sequentially using the scheme illustrated in the diagram. WT indicates the wild type without mutations, while NC represents negative controls without DNA substrate, crRNA, or LbCas12a, respectively. (D-F) SNV identification designed for conventional SARS-CoV-2 *N* targets. (G-I, K) SNV identification designed for suboptimal SARS-CoV-2 *N* targets. NC represents negative controls without DNA substrate, without crRNA, or without LbCas12a, respectively.

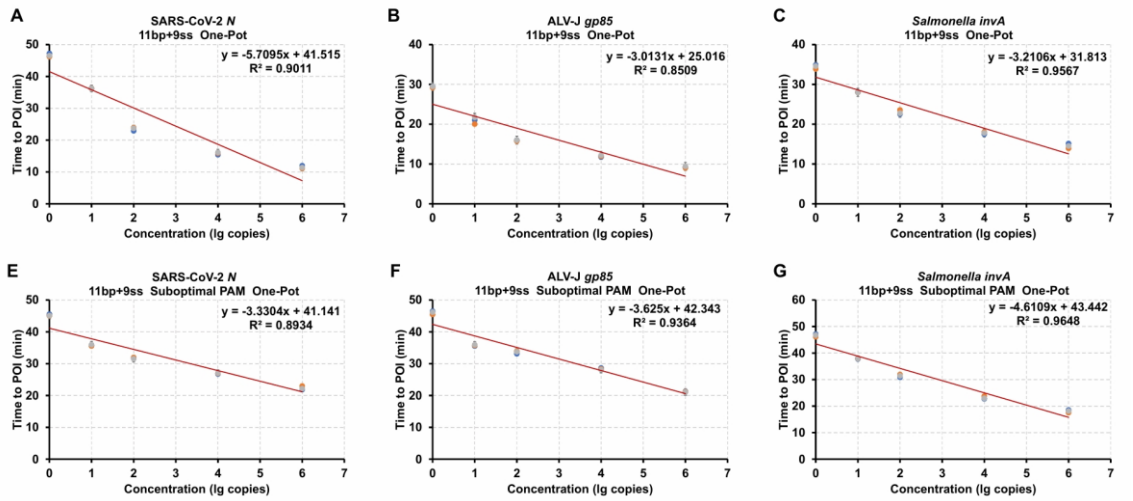


Figure S6. Quantifies the LOD fluorescence values for SARS-CoV-2 *N*, ALV-J *gp85*, and *Salmonella invA*.

(A–C) Linear fitting of the time to point of inflection (POI) versus target concentration using the one-pot 11bp+9ss assay targeting conventional sites. (E–G) Linear fitting of the time to POI versus target concentration using the one-pot 11bp+9ss assay targeting suboptimal sites. All data were presented as mean ± SD (n = 3).

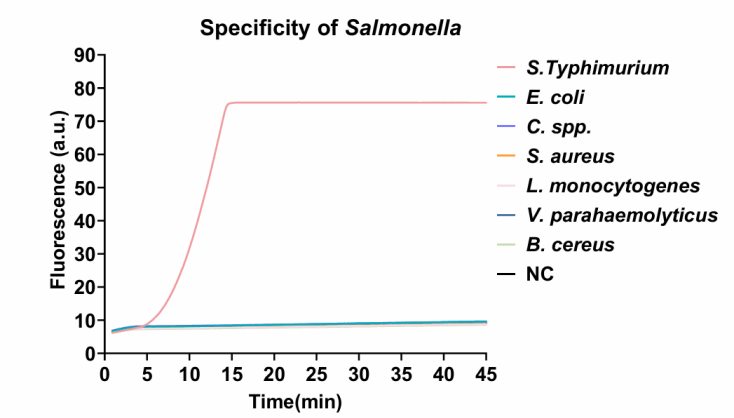


Figure S7. The one-pot method established using the 11bp+9ss strategy provides specific testing for *Salmonella* among common foodborne bacteria.

NC indicates the negative control.

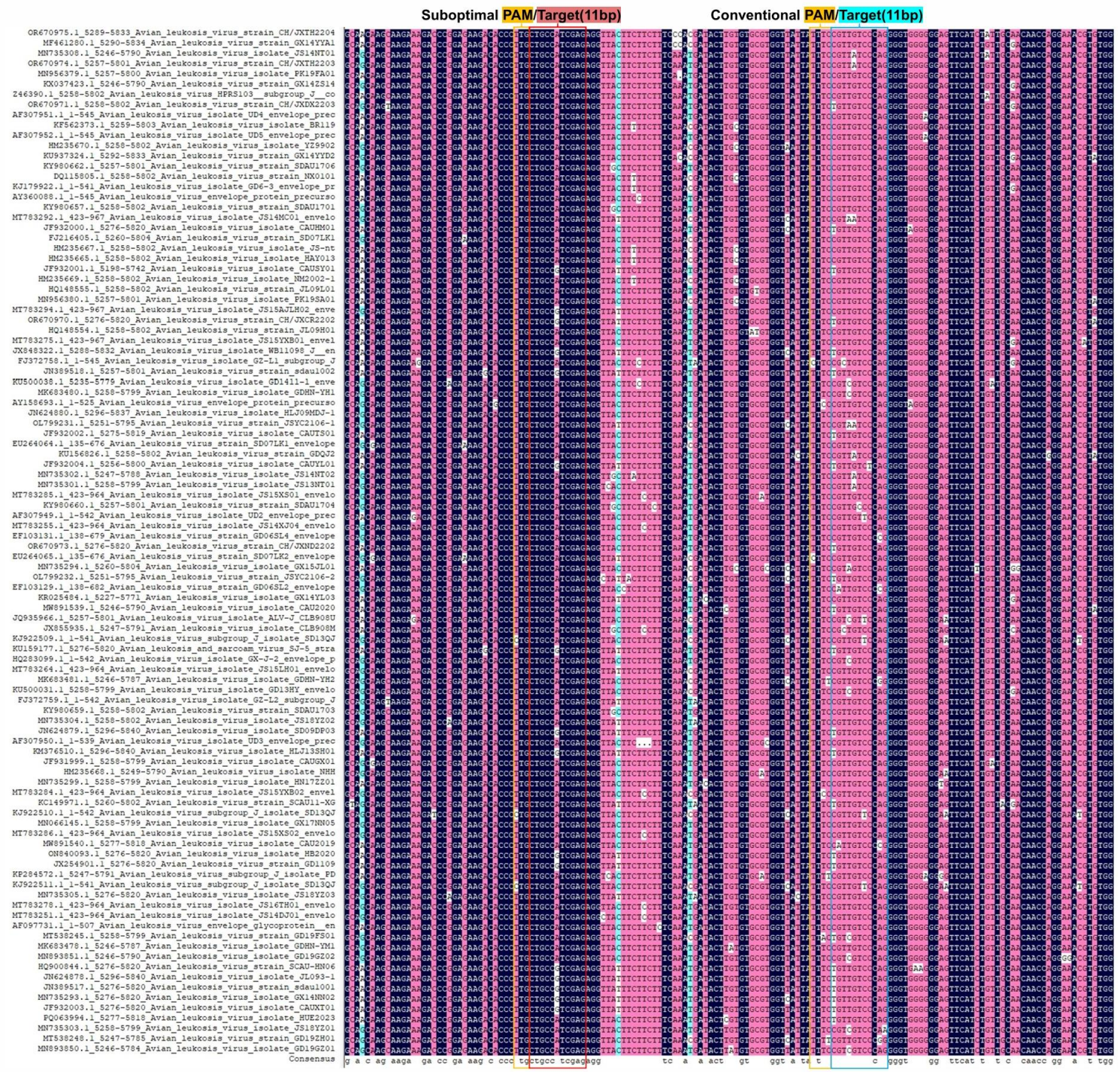


Figure S8. Comparison of the locations and conservation of ALV-J's conventional targets and suboptimal targets.

Conduct a conservation analysis of the ALV-J target using DNAMAN 9.0. The black areas indicate 100% conservation, the pink areas indicate conservation ≥75%, the blue areas indicate conservation ≥50%, and the white areas represent mutation sites. The red box shows suboptimal targets, while the blue box shows conventional targets.

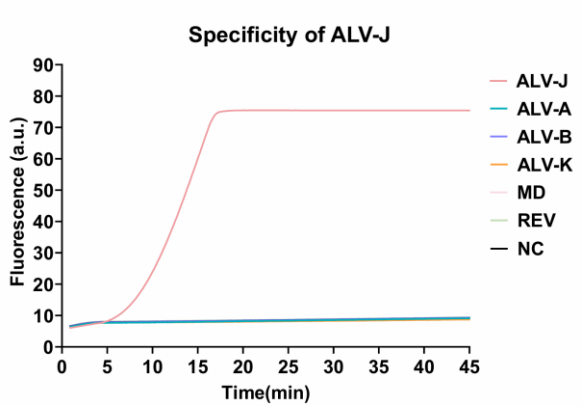


Figure S9. The one-pot method established using the 11bp+9ss strategy was used for specific detection of ALV-J in different subtypes of the virus.

NC indicates the negative control.

Table S1. ssDNA of different lengths and the corresponding spacers.

These are used for preliminary validation of the activation effects of ssDNA of varying lengths on *trans*-cleavage activity. Blue represents the spacer region.

Gene	Object	Length (nt)	Sequences (5'→3')
SARS-CoV-2 *N*	Spacer	20	CCCCCAGCGCUUCAGCGUUC
	ssDNA	6	GAACGC
		7	GAACGCT
		8	GAACGCTG
		9	GAACGCTGA
		10	GAACGCTGAA
		11	GAACGCTGAAG
ALV-J *gp85*	Spacer	20	CGUUGUCCCAGGGGUGGGGG
	ssDNA	6	CCCCCA
		7	CCCCCAC
		8	CCCCCACC
		9	CCCCCACCC
		10	CCCCCACCCC
		11	CCCCCACCCCT
Salmonella invA	Spacer	20	UGGGGUCGUUCUACAUUGAC
	ssDNA	6	GTCAAT
		7	GTCAATG
		8	GTCAATGT
		9	GTCAATGTA
		10	GTCAATGTAG
		11	GTCAATGTAGA
PRV *gE46*	Spacer	20	GGACGACCUCUCCACCGAGG
	ssDNA	6	CCTCGG
		7	CCTCGGT
		8	CCTCGGTG
		9	CCTCGGTGG
		10	CCTCGGTGGA
		11	CCTCGGTGGAG

		12	CCTCGGTGGAGA
		13	CCTCGGTGGAGAG
E. coli rfbE	Spacer	20	UAGCAUUAACUGAUGCUAUA
	ssDNA	6	TATAGC
		7	TATAGCA
		8	TATAGCAT
		9	TATAGCATC
		10	TATAGCATCA
		11	TATAGCATCAG

Table S2. The table contains the primers used for in vitro transcription of the PRV *gE46*.

The T7 promoter is underlined, the scaffold region is green, and the spacer region is blue.

Name	Sequences (5'→3')
PRV *gE46*-F	TAATACGACTCACTATAGGGAATTTCTACTAAGTGTAGATGGACGACCTCTCCACCGAGG
PRV *gE46*-R	CCTCGGTGGAGAGGTCGTCCATCTACACTTAGTAGAAATTCCCTATAGTGAGTCGTATTA

Table S3. crRNAs used for preliminary detection of the effects of different lengths of target dsDNA and ssDNA combinations.

Blue represents the spacer region.

Gene	Sequences (5'→3')
SARS-CoV-2 *N*	AAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC
ALV-J *gp85*	AAUUUCUACUAAGUGUAGAUCGUUGUCCCAGGGGUGGGGG
Salmonella invA	AAUUUCUACUAAGUGUAGAUUGGGGUCGUUCUACAUUGAC
PRV *gE46*	AAUUUCUACUAAGUGUAGAUGGACGACCUCUCCACCGAGG
E. coli rfbE	AAUUUCUACUAAGUGUAGAUUAGCAUUAACUGAUGCUAUA

Table S4. The crRNA involved in the 11bp+9ss strategy.

Blue represents the spacer, while the italicized portion represents the region complementary only to the 9nt ssDNA. The suboptimal target of the SARS-CoV-2 *N* was referenced from Yin [1].

Gene	Sequences (5'→3')
SARS-CoV-2 *N*	AAUUUCUACUAAGUGUAGAUCCCCCAGCGCU*AGUCGCAAG*
ALV-J *gp85*	AAUUUCUACUAAGUGUAGAUCGUUGUCCCAG*AATCTAAAT*
Salmonella invA	AAUUUCUACUAAGUGUAGAUUGGGGUCGUUC*GGUGCCUGA*
SARS-CoV-2 *N* (Suboptimal target)	AAUUUCUACUAAGUGUAGAUUCAUCUGGACU*AUCGCCAAG*
ALV-J *gp85* (Suboptimal target)	AAUUUCUACUAAGUGUAGAUCUGCCAUCGAG*GUUCCGACC*
SARS-CoV-2 *S* (Omicron BA.1)	AAUUUCUACUAAGUGUAGAUCGAUCAUAUAG*AAAGGCUGG*

Table S5. The 9nt ssDNA involved in the conventional 11bp+9ss strategy.

Gene	Sequences (5'→3')
SARS-CoV-2 *N*	CTTGCGACT
ALV-J *gp85*	ATTTAGATT
Salmonella invA	TCAGGCACC
SARS-CoV-2 *N* (Suboptimal target)	CTTGGCGAT
ALV-J *gp85* (Suboptimal target)	GGTCGGAAC
SARS-CoV-2 *S* (Omicron BA.1)	CCAGCCTTT

Table S6. Target dsDNA of varying lengths.

Used for preliminary assessment of the activation effects of target dsDNA of different lengths on *trans*-cleavage activity. Underscores represent the target, green is used to distinguish the shortened part of the target, and gray represents the region of reverse complementation to form the 45bp hairpin.

Gene	Number of target base pairs(bp)	Sequences (5'→3')
SARS-CoV-2 *N*	20	GAAGTCTAATCTTTGCCCCCAGCGCTTCAGCGTTCCCG GTAACAATTGTTACCGGGAACGCTGAAGCGCTGGGGGC AAAGATTAGACTTC
	14	GGAAGTCTAATCTTTGCCCCCAGCGCTAGTATAGGACC GGTAACATGTTACCGGTCCTATACTAGCGCTGGGGGCA AAGATTAGACTTCC
	13	GGAAGTCTAATCTTTGCCCCCAGCGCTAGCATAGGACC GGTAACATGTTACCGGTCCTATGCTAGCGCTGGGGGCA AAGATTAGACTTCC
	12	AGGAAGTCTAATCTTTGCCCCCAGCGCTATCATAGGACC GGTAACGTTACCGGTCCTATGATAGCGCTGGGGGCAAA GATTAGACTTCCT
	11	AGGAAGTCTAATCTTTGCCCCCAGCGCTGTCATAGGAC CGGTAACGTTACCGGTCCTATGACAGCGCTGGGGGCAA AGATTAGACTTCCT
	10	AGGAAGTCTAATCTTTGCCCCCAGCGCCGTCATAGGAC CGGTAACGTTACCGGTCCTATGACGGCGCTGGGGGCAA AGATTAGACTTCCT
	9	TAGGAAGTCTAATCTTTGCCCCCAGCGACGTCATAGGA CCGGTAATTACCGGTCCTATGACGTCGCTGGGGGCAAA GATTAGACTTCCTA
ALV-J *gp85*	20	CGTGGTTATTATTTCCGTTGTCCCAGGGGTGGGGGGAG TTCATCTAGATGAACTCCCCCCACCCCTGGGACAACGG AAATAATAACCACG

	14	GTGTGCGTGGTTATTATTTCCGTTGTCCCAGGGGCTAAATGAGTTAACTCATTTAGCCCCTGGGACAACGGAAATAATAACCACGCACAC
	13	GTGTGCGTGGTTATTATTTCCGTTGTCCCAGGGACTAAATGAGTTAACTCATTTAGTCCCTGGGACAACGGAAATAATAACCACGCACAC
	12	GTGTGCGTGGTTATTATTTCCGTTGTCCCAGGAACTAAATGAGTTAACTCATTTAGTTCCTGGGACAACGGAAATAATAACCACGCACAC
	11	GTGTGCGTGGTTATTATTTCCGTTGTCCCAGAAACTAAATGAGTTAACTCATTTAGTTTCTGGGACAACGGAAATAATAACCACGCACAC
	10	GTGTGCGTGGTTATTATTTCCGTTGTCCCATAAACTAAATGAGTTAACTCATTTAGTTTATGGGACAACGGAAATAATAACCACGCACAC
	9	GTGTGCGTGGTTATTATTTCCGTTGTCCCGTAAACTAAATGAGTTAACTCATTTAGTTTACGGGACAACGGAAATAATAACCACGCACAC
Salmonella invA	20	TGGCGATATTGGTGTTTATGGGGTCGTTCTACATTGACAGAATCCGGATTCTGTCAATGTAGAACGACCCCATAAACACCAATATCGCCA
	14	GCGATATTGGTGTTTATGGGGTCGTTCTACGCCTGAAGAATCCTCGAGGATTCTTCAGGCGTAGAACGACCCCATAAACACCAATATCGC
	13	GCGATATTGGTGTTTATGGGGTCGTTCTATGCCTGAAGAATCCTCGAGGATTCTTCAGGCATAGAACGACCCCATAAACACCAATATCGC
	12	GGCGATATTGGTGTTTATGGGGTCGTTCTGTGCCTGAAGAATCCTAGGATTCTTCAGGCACAGAACGACCCCATAAACACCAATATCGCC

	11	GGCGATATTGGTGTTTATGGGGTCGTTCGGTGCCTGAAG AATCCTAGGATTCTTCAGGCACCGAACGACCCCATAAA CACCAATATCGCC
	10	TGGCGATATTGGTGTTTATGGGGTCGTTAGGTGCCTGAA GAATCCGGATTCTTCAGGCACCTAACGACCCCATAAAC ACCAATATCGCCA
	9	TGGCGATATTGGTGTTTATGGGGTCGTCAGGTGCCTGAA GAATCCGGATTCTTCAGGCACCTGACGACCCCATAAAC ACCAATATCGCCA
PRV *gE46*	20	ATCGTCGTCGGCCTCGGTGGAGAGGTCGTCCCAAACCT CGGCCGATCGGCCGAGGTTTGGGACGACCTCTCCACCG AGGCCGACGACGAT
	19	TCGTCGTCGGTCTCGGTGGAGAGGTCGTCCCAAACCTC GGCCGAGCTCGGCCGAGGTTTGGGACGACCTCTCCAC CGAGACCGACGACGA
	18	TCGTCGTCGGTTTCGGTGGAGAGGTCGTCCCAAACCTC GGCCGAGCTCGGCCGAGGTTTGGGACGACCTCTCCAC CGAAACCGACGACGA
	17	CGTCGTCGGTTCCGGTGGAGAGGTCGTCCCAAACCTCG GCCGAGGCCTCGGCCGAGGTTTGGGACGACCTCTCCA CCGGAACCGACGACG
	16	CGTCGTCGGTTCAGGTGGAGAGGTCGTCCCAAACCTCG GCCGAGGCCTCGGCCGAGGTTTGGGACGACCTCTCCA CCTGAACCGACGACG
	15	GTCGTCGGTTCATGTGGAGAGGTCGTCCCAAACCTCGG CCGAGGGCCCTCGGCCGAGGTTTGGGACGACCTCTCC ACATGAACCGACGAC
	14	GTCGTCGGTTCATTTGGAGAGGTCGTCCCAAACCTCGG CCGAGGGCCCTCGGCCGAGGTTTGGGACGACCTCTCC AAATGAACCGACGAC

	13	TCGTCGGTTCATTCGGAGAGGTCGTCCCAAACCTCGGC CGAGGGATCCCTCGGCCGAGGTTTGGGACGACCTCTCC GAATGAACCGACGA
	12	TCGTCGGTTCATTCTGAGAGGTCGTCCCAAACCTCGGC CGAGGGATCCCTCGGCCGAGGTTTGGGACGACCTCTCA GAATGAACCGACGA
	11	CGTCGGTTCATTCTTAGAGGTCGTCCCAAACCTCGGCC GAGGGACGTCCCTCGGCCGAGGTTTGGGACGACCTCTA AGAATGAACCGACG
	10	CGTCGGTTCATTCTTGGAGGTCGTCCCAAACCTCGGCC GAGGGACGTCCCTCGGCCGAGGTTTGGGACGACCTCC AAGAATGAACCGACG
	9	GTCGGTTCATTCTTGTAGGTCGTCCCAAACCTCGGCCG AGGGACTAGTCCCTCGGCCGAGGTTTGGGACGACCTAC AAGAATGAACCGAC
	8	GTCGGTTCATTCTTGTGGGTCGTCCCAAACCTCGGCCG AGGGACTAGTCCCTCGGCCGAGGTTTGGGACGACCCA CAAGAATGAACCGAC
	7	TCGGTTCATTCTTGTGTGTCGTCCCAAACCTCGGCCGA GGGACTCGAGTCCCTCGGCCGAGGTTTGGGACGACAC ACAAGAATGAACCGA
E. coli rfbE	20	CTGTGTATTTTATAGCATTAACTGATGCTATATATGT CAGTGTTGCAACACTGACATATATAGCATCAGTTA ATGCTATAAAATACACAG
	14	CTCCTGTGTATTTTATAGCATTAACTGATTAGCGCT ATGTCAGTGCACTGACATAGCGCTAATCAGTTAAT GCTATAAAATACACAGGAG
	13	GCTCCTGTGTATTTTATAGCATTAACTGACTAGCGC TATGTCAGTACTGACATAGCGCTAGTCAGTTAATG CTATAAAATACACAGGAGC

	12	GCTCCTGTGTATTTTATAGCATTAACTGGCTAGCGC TATGTCAGTACTGACATAGCGCTAGCCAGTTAATG CTATAAAATACACAGGAGC
	11	TGGCTCCTGTGTATTTTATAGCATTAACTTGCTAGC GCTATGTCATGACATAGCGCTAGCAAGTTAATGCT ATAAAATACACAGGAGCCA
	10	TGGCTCCTGTGTATTTTATAGCATTAACCTGCTAGC GCTATGTCATGACATAGCGCTAGCAGGTTAATGCT ATAAAATACACAGGAGCCA
	9	TGGCTCCTGTGTATTTTATAGCATTAATCTGCTAGC GCTATGTCATGACATAGCGCTAGCAGATTAATGCT ATAAAATACACAGGAGCCA

Table S7. The table contains 45bp hairpins with canonical or suboptimal PAM as dsDNA activators.

Underscores represent the target, and gray represents the region of reverse complementation to form the 45bp hairpin.

Gene	Sequences (5'→3')
SARS-CoV-2 *N*	GAAGTCTAATCTTTGCCCCCAGCGCTTCAGCGTTCCCGGTAACAATTGT TACCGGGAACGCTGAAGCGCTGGGGGCAAAGATTAGACTTC
	GAAGTCTAATCGTTGCCCCCAGCGCTTCAGCGTTCCCGGTAACAATTGT TACCGGGAACGCTGAAGCGCTGGGGGCAACGATTAGACTTC
	GAAGTCTAATCTTGGCCCCCAGCGCTTCAGCGTTCCCGGTAACAATTGT TACCGGGAACGCTGAAGCGCTGGGGGCCAAGATTAGACTTC
	GAAGTCTAATCTCTGCCCCCAGCGCTTCAGCGTTCCCGGTAACAATTGT TACCGGGAACGCTGAAGCGCTGGGGGCAGAGATTAGACTTC
ALV-J *gp85*	AGATGAACTCCCCCCACCCCTGGGACAACGGAAATAATAACCACGCG TGGTTATTATTTCCGTTGTCCCAGGGGTGGGGGGAGTTCATCT
	AGATGAACTCCCCCCACCCCTGGGACAACGCAACTAATAACCACGCGT GGTTATTAGTTGCGTTGTCCCAGGGGTGGGGGGAGTTCATCT
	AGATGAACTCCCCCCACCCCTGGGACAACGCCAATAATAACCACGCGT GGTTATTATTGGCGTTGTCCCAGGGGTGGGGGGAGTTCATCT

	AGATGAACTCCCCCCACCCCTGGGACAACGCAGATAATAACCACGCGT GGTTATTATCTGCGTTGTCCCAGGGGTGGGGGGAGTTCATCT
Salmonella invA	TGGCGATATTGGTGTTTATGGGGTCGTTCTACATTGACAGAATCC GGATTCTGTCAATGTAGAACGACCCCATAAACACCAATATCGCCA
	TGGCGATATTGGTGGTTGTGGGGTCGTTCTACATTGACAGAATCCGGAT TCTGTCAATGTAGAACGACCCCACAACCACCAATATCGCCA
	TGGCGATATTGGTGTTGGTGGGGTCGTTCTACATTGACAGAATCCGGAT TCTGTCAATGTAGAACGACCCCACCAACACCAATATCGCCA
	TGGCGATATTGGTGTCTGTGGGGTCGTTCTACATTGACAGAATCCGGAT TCTGTCAATGTAGAACGACCCCACAGACACCAATATCGCCA

Table S8 ssDNA, spacer, and the corresponding target sequence used for SNV identification.

The suboptimal target of the SARS-CoV-2 *N* was referenced from Yin[1]. CT and ST are used to distinguish between conventional targets and suboptimal targets. Red represents PAM. Green indicates mismatched points. The underline indicates that this section is complementary only to ssDNA. Bases highlighted in yellow represent SNV used to differentiate between Omicron BA.1 and BA.2.

Gene	Name	Sequences (5'→3')
SARS-CoV-2 *N*	CT-target	TTTGCCCCCAGCGCTTCAGCGTTC
	CT-spacer	CCCCCAGCGCUGACUAUGGA
	CT-9ss	TCCATAGTC
	CT-8ss	TCCATAGT
	CT-9ssMis	TCCAGAGTC
	ST-target	TTGGTCATCTGGACTGCTATTGGT
	ST-spacer	UCAUCUGGACUAUGCGCAUC
	ST-9ss	GATGCGCAT
	ST-8ss	GATGCGCA
	ST-9ssMis	GATGAGCAT
	ST-8ssMis	GATGAGCA
SARS-CoV-2 *S*	BA.1-target	TTTACGATCATATAGTTTCCGACC
	BA.1-spacer	CGAUCAUAUAGAAAGGCUGG

	BA.1-9ss	CCAGCCTTT
	BA.1-8ss	CCAGCCTT
	BA.1-9ssMis	CCAGACTTT

Table S9. 45bp hairpins used for the identification of SNVs.

CT and ST are used to distinguish between conventional targets and suboptimal targets. The underline indicates the target. The green letters indicate mutation sites. Bases highlighted in yellow represent SNV used to differentiate between Omicron BA.1 and BA.2. The gray letters indicate the reverse complementary section within the 45bp hairpin.

Gene	Name	Sequences (5'→3')
SARS-CoV-2 *N*	CT-WT	ATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAAGCGCTGGG GGCAAATTGTGCAAT
	CT-MT1	ATTGCACAATTTGACCCCAGCGCTTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAAGCGCT GGGGTCAAATTGTGCAAT
	CT-MT2	ATTGCACAATTTGCACCCAGCGCTTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAAGCGCT GGGTGCAAATTGTGCAAT
	CT-MT6	ATTGCACAATTTGCCCCCGGCGCTTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAAGCGCC GGGGGCAAATTGTGCAAT
	CT-MT10	ATTGCACAATTTGCCCCCAGCGATTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAATCGCTG GGGGCAAATTGTGCAAT
	CT-MT11	ATTGCACAATTTGCCCCCAGCGCCTCAGCGTTCTTCGG AATGTCGCGACATTCCGAAGAACGCTGAGGCGCT GGGGGCAAATTGTGCAAT
	ST-WT	GTAGCCAATTTGGTCATCTGGACTGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCAGTCCAG ATGACCAAATTGGCTAC

	ST-MT1	GTAGCCAATTTGGCCATCTGGACTGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCAGTCCAG ATGGCCAAATTGGCTAC
	ST-MT2	GTAGCCAATTTGGTAATCTGGACTGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCAGTCCAG ATTACCAAATTGGCTAC
	ST-MT6	GTAGCCAATTTGGTCATCCGGACTGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCAGTCCGG ATGACCAAATTGGCTAC
	ST-MT10	GTAGCCAATTTGGTCATCTGGAATGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCATTCCAGA TGACCAAATTGGCTAC
	ST-MT11	GTAGCCAATTTGGTCATCTGGACGGCTATTGGTGTTAAT TGGAACGTTCCAATTAACACCAATAGCCGTCCAGATGA CCAAATTGGCTAC
SARS-CoV-2 *S*	Omicron BA.1	TTACTTTCCTTTACGATCATATAGTTTCCGACCCACTTAT GGTGTACACCATAAGTGGGTCGGAAACTATATGATCGT AAAGGAAAGTAA
	Omicron BA.2	TTACTTTCCTTTACGATCATATGGTTTCCGACCCACTTAT GGTGTACACCATAAGTGGGTCGGAAACCATATGAT CGTAAAGGAAAGTAA

Table S10. Primers for RPA.

Primer name	Length (bp)	Sequences (5'→3')
SARS-CoV-2 *N* RPA-F	189	TACCTGTATAGATTGTTTAGGAAGTCTAAT
SARS-CoV-2 *N* RPA-R		AAAAGAAAGTACTACTACTCTGTATGGTTG
ALV-J *gp85* RPA-F	148	CAACAAGCAAGAAAGACCCGGAGAAGACAC
ALV-J *gp85* RPA-R		CACACGTTTCCTGGTTGTTGCAATAGATG

Salmonella invA RPA-F	200	GTCATTCCATTACCTACCTATCTGGTTGATTTCC
Salmonella invA RPA-R		GCATCGGCTTCAATCAAGATAAGACGGCTGGT

Table S11. Primers for PCR.

The numerical values in the primer names represent approximate ranges and are only used to differentiate primers of different lengths. The actual length of the primers should be based on the annotation in the figures.

Primer name	Tm (℃)	Sequences (5'→3')
200-PCR-F	60	PCR was run directly using RPA primers, and the primer sequences were the same as in Supplementary Table.
200-PCR-R		
750-PCR-F		GGATGAGGTGACTAAGAAAG
750-PCR-R		CGAACCAAAGGTAACACACG
3000-PCR-F		TTCATTCAGCTCCGGTTCCC
3000-PCR-F		GCGTCAGACCCCGTAGAAAA

Table S12. qPCR and RT-qPCR primers.

Object	Length (bp)	Name	Sequences (5'→3')
Salmonella	260	qPCR-F	CTTTGGTCGTAAAATAAGGCG
		qPCR-R	TGCCCAAAGCAGAGAGATTC
ALV-J	253	RT-qPCR-F	GGATGAGGTGACTAAGAAAG
		RT-qPCR-R	ATAATCGTACATAGCTTCGTCTA

References

[1] Shuhan L, Xiaohan T, Yang H, et al, Fast and sensitive detection of SARS-CoV-2 RNA using suboptimal protospacer adjacent motifs for Cas12a.[J], Nature biomedical engineering, 2022, 6(3): 286-297