

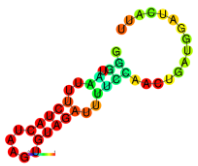
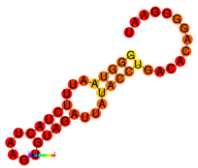
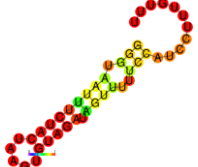
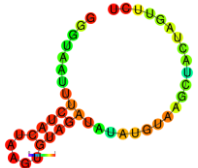
Table S1. The primer sequences employed in this study.

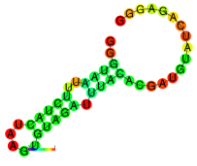
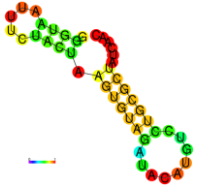
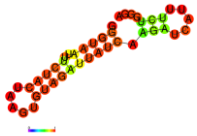
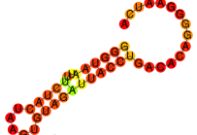
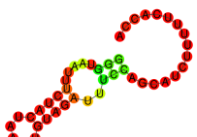
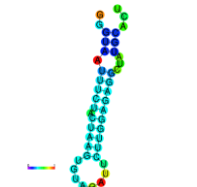
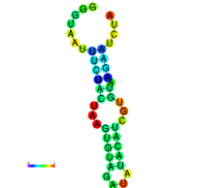
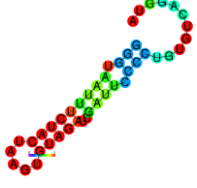
RPA primer region 1-<i>plc</i>	
F1	CTTAGAGATTTTAAAAGAGAACATGCATGAG
F2	CATGCATGAGCTTCAATTAGGTTCTAC
F3	CAGATTATGATAAGAACGCCTATGATC
F4	GATAAGAACGCCTATGATCTATATCAAGATC
R1	CTCAAACCTTAACATGTCCTGCGCTATCAACG
R2	AATCCTCATTAGTTTTGCAACCTGCTGTG
R3	CTGTTCTTTTCTTTCCTCTGCAAAAGTCTC
R4	CCTCATTAGTTTTGCAACCTGCTGTGTTTA
R5	TCTGCAAAAGTCTCAAACCTTAACATGTC
RPA primer region 2-<i>plc</i>	
F5	CTAGCATGAGTCATAGTTGGGATGATTGG
F6	CATAGTTGGGATGATTGGGACTATGCAGCA
R6	TTGAAAGTATAAGTATCTTTACTTCCAGTC
R7	AATCATTTCCTGGGTTGTCCATTTCCTATTC
RPA primer region 1-<i>cpe</i>	
F1	CAGTTGGATTTACTTCTGAATTTATACAAGC
F2	CCTAATGAAACAGGTACCTTTAGCCAATC
F3	CAGGTACCTTTAGCCAATCATTAATAAATC
R1	CTGCAGATGTTTTACTAAGCCATATTCCTG
R2	CCTTGATCAATATTTCTTAAGCTATCTGC
R3	CCTAAGCTATCTGCAGATGTTTTACTAAGCC
RPA primer region 2-<i>cpe</i>	
F4	TCCAGCTGGTAATTTATATGATTGGCGTTC
F5	AACTGATGCATTAAACTCAAATCCAGCTGG
F6	GCTGCTGCTACAGAAAGATTAAATTTAACTG
R4	CCATCACCTAAGGACTGTTCTAATTTCACTAG

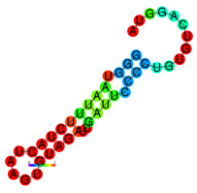
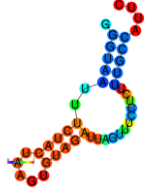
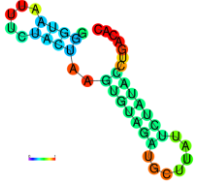
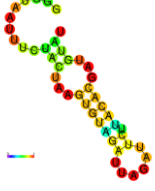
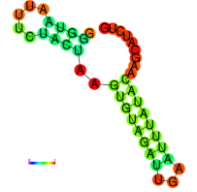
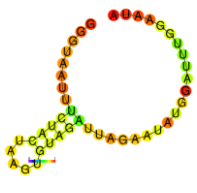
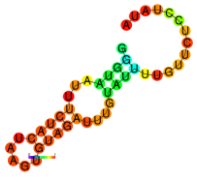
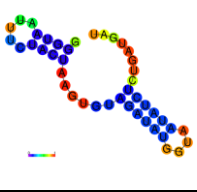
R5	TCCAGCATCTAAGCTTATATCAACATAATG
R6	TTACAAGAACATATTGTCCAGCATCTAAGC
qPCR-<i>plc</i>	
F	CCGTTGATAGCGCAGGACA
R	CCCAACTATGACTCATGCTAGCA
qPCR-<i>cpe</i>	
F	GGTTCATTAATTGAAACTGGTG
R	AACGCCAATCATATAAATTACCAG
PCR primers-<i>plc</i> gene standard plasmid	
F	TGGGATGGAAAAATTGATGGAACA
R	TTTATATTATAAGTTGAATTCCTGAA

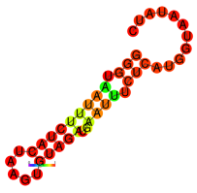
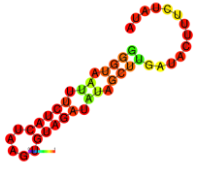
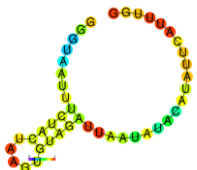
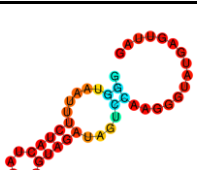
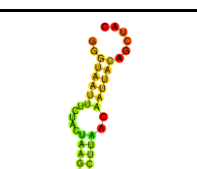
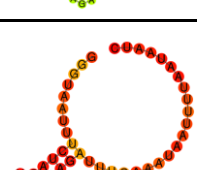
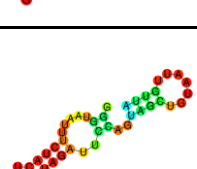
The sequences of the final selected RPA primers are highlighted in bold in Table S1 and also listed in Table 1 of the main text.

Table S2. The study presents the PAM, sequence, and structural diagram of the crRNA

<i>plc</i> gene			
number	PAM	sequences	structure
1	ATTC	GGGUAUUUCUACUAAGUGUAGAUUUUCCA ACUGAUGGAUCAUU	
2	ATTC	GGGUAUUUCUACUAAGUGUAGAUUAUACC UGACACAGGGGAAU	
3	CTTG	GGGUAUUUCUACUAAGUGUAGAUAGUUU UCCAUCCUUUGUUU	
4	GTTG	GGGUAUUUCUACUAAGUGUAGAUUAUAG UAAGCUACUAGUUCU	

5	ATTC	GGGUAUUUCUACUAAGUGUAGAUUUACAC GAUGUAUCAGAGGG	
6	CTTA	GGGUAUUUCUACUAAGUGUAGAUACAUG UCCUGCGCUAUCAAC	
7	TCTA	GGGUAUUUCUACUAAGUGUAGAUUAUCA AGAUCAUUUCUGGGA	
8	TCTA	GGGUAUUUCUACUAAGUGUAGAUUACCUG ACACAGGGGAAUCA	
9	TCTG	GGGUAUUUCUACUAAGUGUAGAUUCCAG CAUCUUUUUCACCA	
10	TCTA	GGGUAUUUCUACUAAGUGUAGAUUCUUGG AGAGGCUAUGCACU	
11	TCTG	GGGUAUUUCUACUAAGUGUAGAUAUACA UCGUGUAAGAAUCUA	
12	TTTG	GGGUAUUUCUACUAAGUGUAGAUUGAUU CCCCUGUGUCAGGUA	

13	TTTC	GGGUAUUUCUACUAAGUGUAGAUCUCUUU GCCAUUCAUAUCUA	
14	TTTA	GGGUAUUUCUACUAAGUGUAGAUUAGUU UCCUCUUUGCCAUUC	
15	TTTA	GGGUAUUUCUACUAAGUGUAGAUGCUUA UUCUAUACCUGACAC	
16	TTTA	GGGUAUUUCUACUAAGUGUAGAUUAGAU UCUACACGAUGUAU	
<i>cpe gene</i>			
1	CTTC	GGGUAUUUCUACUAAGUGUAGAUUGAAU UUAUACAAGCAUCUG	
2	TCTG	GGGUAUUUCUACUAAGUGUAGAUUAGAA UAUGGAUUUGGAAUA	
3	TCTA	GGGUAUUUCUACUAAGUGUAGAUUUGUA UUUUGUUCUCCUAUA	
4	TCTC	GGGUAUUUCUACUAAGUGUAGAU AUGGU AAUAUCUCUGAUGAU	

5	ATTA	GGGUAUUUCUACUAAGUGUAGAUGAAUU UCUCAUGGUAUAUC	
6	TCTA	GGGUAUUUCUACUAAGUGUAGAUUAGC UUGAUACUUUCUAUA	
7	CTTA	GGGUAUUUCUACUAAGUGUAGAUUAAUA UACAUAUUCAUUUGG	
8	TTTA	GGGUAUUUCUACUAAGUGUAGAUUAUGA UUGGCGUUCUUCUAA	
9	TTTG	GGGUAUUUCUACUAAGUGUAGAUAGUCCA AGGGUAUGAGUUAG	
10	TTTA	GGGUAUUUCUACUAAGUGUAGAUACACUUA ACAAUUACAGCUAC	
11	TTTA	GGGUAUUUCUACUAAGUGUAGAUUUCAA AUAAUUUUAAUAAUC	
12	TTTG	GGGUAUUUCUACUAAGUGUAGAUUCCAGU AGCUGAAUUGUUA	

The sequences of the final selected crRNAs (spacer and PAM) are highlighted in bold in Table S2 and also listed in Table 1 of the main text.

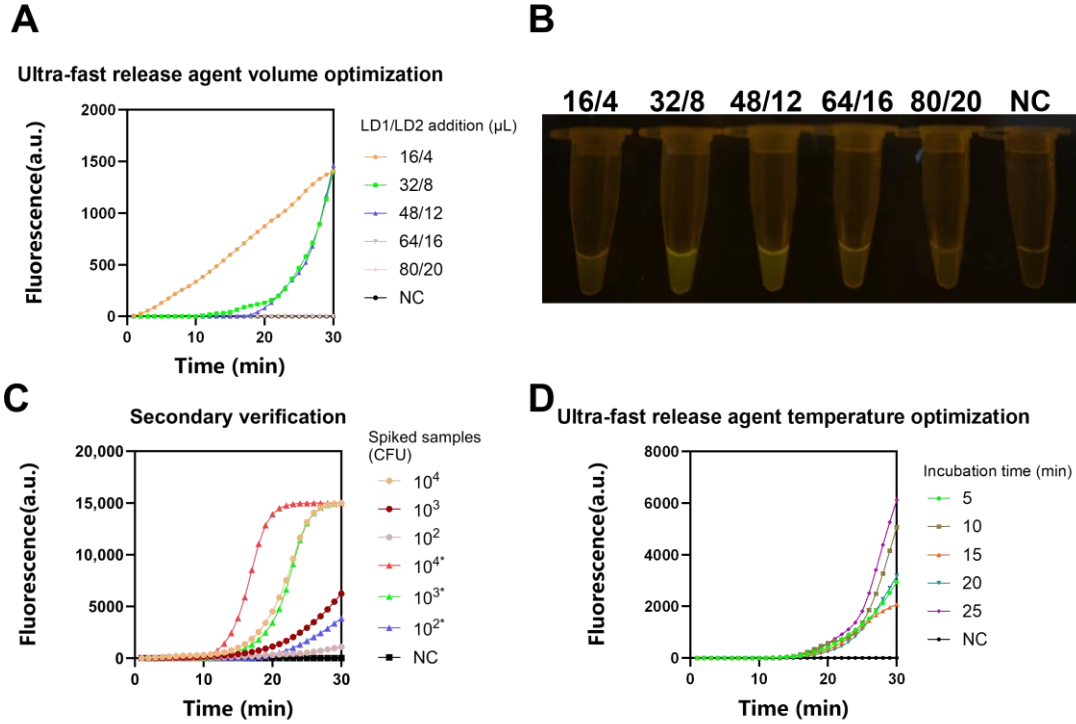


Figure S1. Optimization of the ultra-fast release agent addition. The ultra-fast release agent can extract DNA from homogenized tissue supernatant using 16 μL of LD-1 and 4 μL of LD-2. This reagent was used in this study to directly extract DNA from finely chopped meat tissue. To 20 mg of muscle tissue, 100 μL of purified water, 32 μL of LD-1, and 8 μL of LD-2 were added, followed by incubation at 40°C for 5 min. The supernatant was used as the target DNA for detection. A: The DNA samples, to which 10^2 CFU of *C. perfringens* was added, were extracted using varying volumes of LD-1/LD-2. A more significant increase in fluorescence signal was observed when the volumes of LD-1 and LD-2 were doubled and tripled. B: After incubation in a 37°C metal bath for an additional 10 min, the reaction tubes with 32/8 and 48/12 demonstrated brighter fluorescence. C: Samples containing 10^4 , 10^3 , and 10^2 CFU of *C. perfringens* were extracted using either 16/4 or 32/8 volumes of the ultra-fast release agent. * Denotes the 32/8 LD-1/LD-2. Using 32/8 of the ultra-fast release agent for sample DNA extraction resulted in an earlier peak time and a more robust fluorescence signal in the RPA-Cas12a-*plc* reaction. D: Samples containing 10^2 CFU of *C. perfringens* were extracted with 32/8 of LD-1/LD-2, incubated for 5, 10, 15, 20, and 25 min, and then subjected to the RPA-Cas12a-*plc* reaction. Increasing the incubation time did not result in an earlier fluorescence signal.