

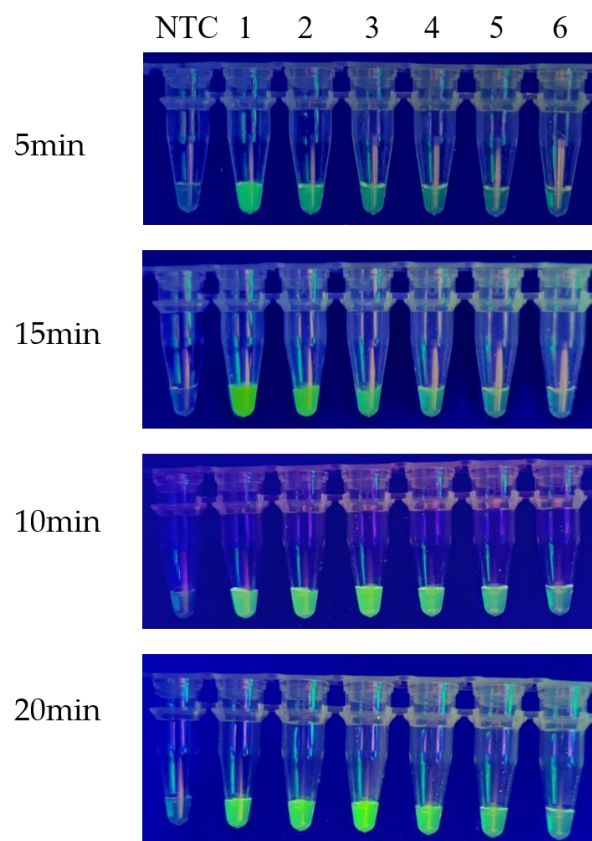
## Supplementary data

**Table S1.** The list of all used sequences in this study.

Primer name	Sequence (5'-3')
ssDNA-FQ reporter	FAM-TTTTTTTTTTTT-BHQ1
crRNA-NOS 1	UAAUUUCUACUCUUGUAGAUAAUACGCGAUAGAAAACAAA
crRNA-NOS 2	UAAUUUCUACUCUUGUAGAUUCUGUUGAAUUACGUUAAGCA
crRNA-NOS 3	UAAUUUCUACUCUUGUAGAUUGGCAAUAAAGUUUCUUAAGA
NOS-QF_SN	TTGGCAATAAAGTTTCTTAAGATTGAAT
NOS-QR_SN	ACATGCTTAACGTAATTCAACAGAAATT
NOS-QP_SN	6-FAM-CTGTTGCCGGTCTTGCGATGATTATCAT-BHQ-1
NOS-F1	GAATCCTGTTGCCGGTCTTG
NOS-R1	TTATCCTAGTTTGCGCGCTA
NOS-F2	ATCGTTCAAACATTTGGCA
NOS-R2	ATTGCGGGACTCTAATCATA

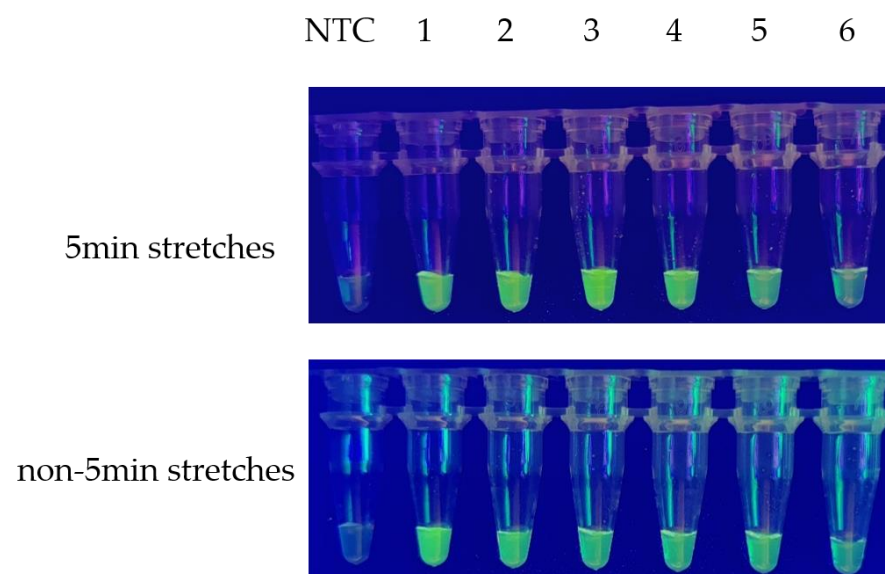
**Table S2.** Ct values of qPCR for T-nos detection from 11 soybean DNA samples.

Samples	NTC	GM1	GM2	GM3	GM4	GM5	GM6
CT value	-	24.37±0.02	30.28±0.01	31.88±0.11	30.69±0.1	32.28±0.04	24.83±0.04
Sample	GM7	Non-GM1	Non-GM2	Non-GM3	Non-GM4		
CT value	29.53±0.05	-	-	-	-		

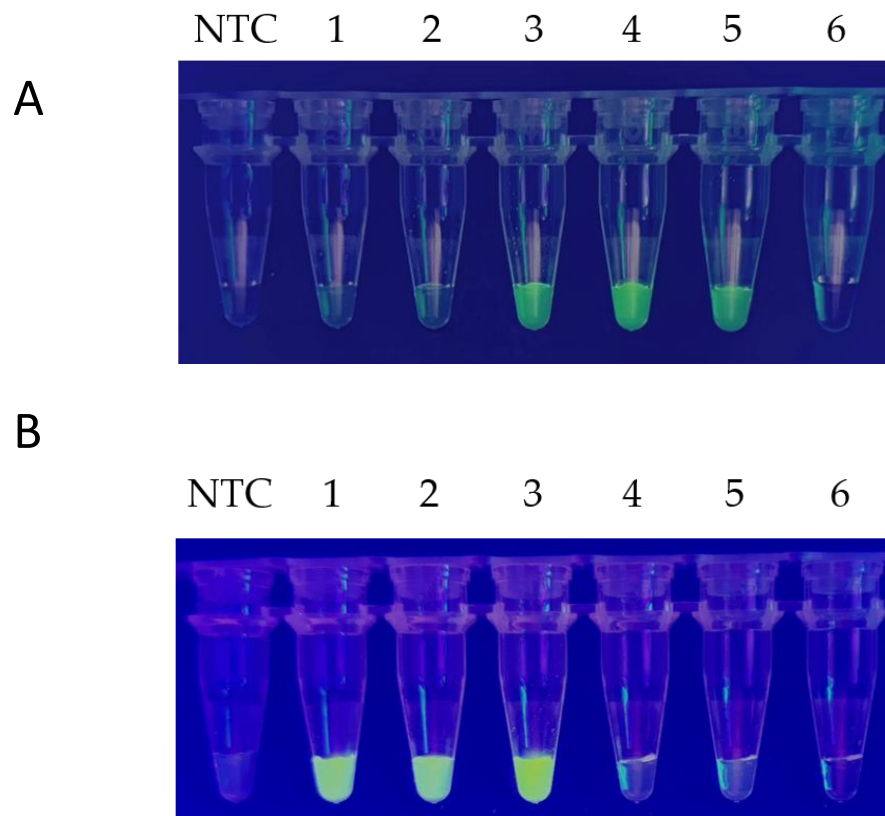


**Figure S1.** Endpoint fluorescence graphs for the T-nos detection with various incubation time (5, 10, 15 and 20 min) at 37 °C. No obvious change in visual fluorescence after incubation for 15 minutes or 20 minutes.

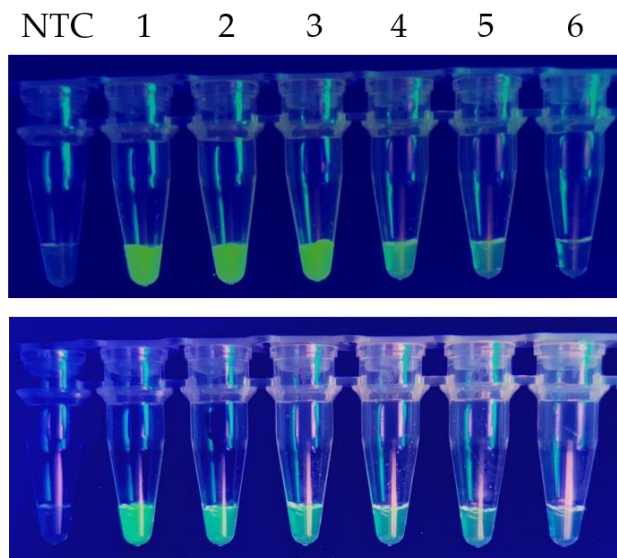
NTC, no template control; Lanes 1–6,  $9 \times 10^4$ ,  $1.8 \times 10^4$ ,  $3.6 \times 10^3$ ,  $7.2 \times 10^2$ , 144, and 29 copies/ $\mu$ L plasmid.



**Figure S2.** Effect of PCR with or without the final 5 min extension on CRISPR reaction. NTC, no template control; Lanes 1–6,  $9 \times 10^4$ ,  $1.8 \times 10^4$ ,  $3.6 \times 10^3$ ,  $7.2 \times 10^2$ , 144, and 29 copies/ $\mu\text{L}$  plasmid.



**Figure S3.** Specificity evaluation of the CRISPR/Cas12a assay for T-nos detection.  
A: 1-2: CaMV 35S promoter, T-35S terminator; 3-5: Three transgenic corn containing T-nos samples; 6: non-NOS GM soybean (MON89788).  
B: 1-3: Three different DNA concentrations of GM soybean ZUTS-33; 4-6: Three different DNA concentrations of non-GM soybeans.



**Figure S4.** Two other repetitions based on sensitivity test.