Electronic Supplementary Information for

An instant, visual and instrument-free method for on-site screening of GTS 40-3-2 soybean based on body-heat triggered recombinase polymerase amplification

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Materials and reagents

In total, we have tested 16 kinds of samples, including transgenic soybean GTS 40-3-2 (provided by College of Life Sciences, Zhejiang University, Hangzhou, China); transgenic soybean DP 305423 and DP 356043 (purchased from DuPont Pioneer Hi-bred International, Inc., MA, USA); transgenic rice LL 601 (purchased from Monsanto Co., MO, USA); transgenic rice TT51-1 (obtained from Huazhong Agricultural University, Wuhan, China); transgenic maize MON 810 (purchased from Monsanto Co. MO, USA); non-transgenic soybean, maize and rice (purchased from local market, Hangzhou, China); oilseed rape, potato, beet, peanut, sugar cane, radish, pumpkin (purchased from the local market, Hangzhou, China).

Reagents of sodium hydroxide (NaOH) and agarose were both purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). TIANamp plant DNA kit was purchased from Tiangen Biotech Co., Ltd. (Beijing, China) for standard DNA extraction. TaKaRa Taq™ Hot Start amplification kit was purchased from Takara Bio Inc. (Dalian, China) for PCR amplification. TwistAmp® Basic kit was purchased from TwistDx Ltd. (Hertfordshire, AL UK) for RPA amplification. SYTO 9 was purchased from Thermo Fisher Scientific Inc. (Waltham, MA USA). SYBR Green I was purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Figure S1

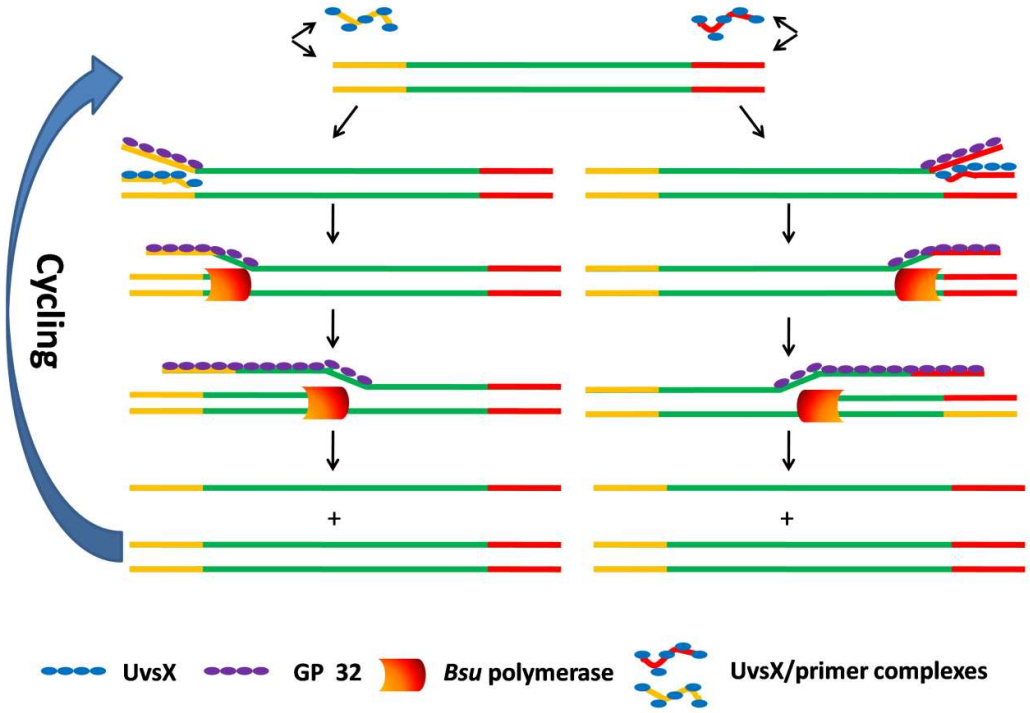


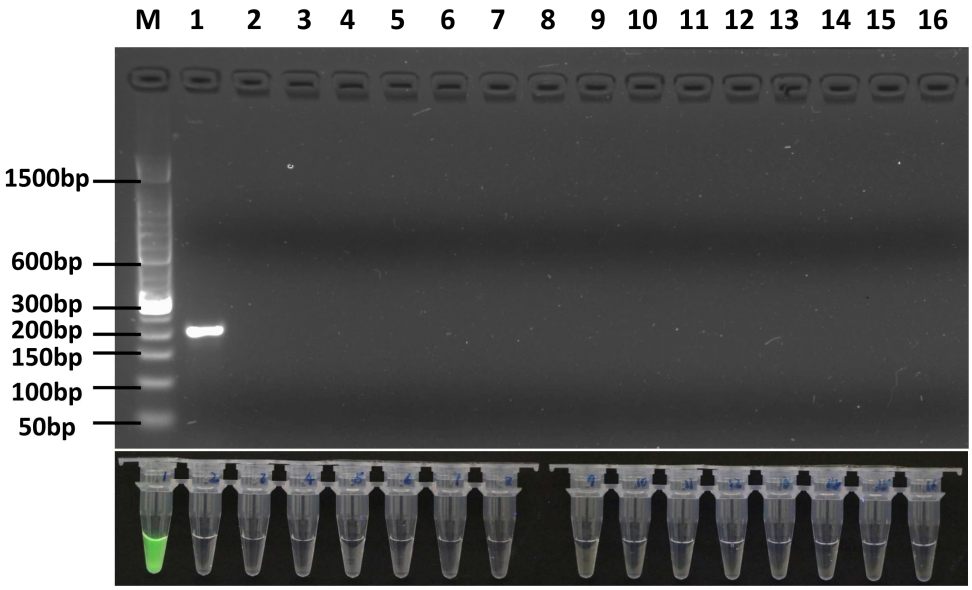
Figure S1 Schematic of RPA amplification. The template DNA is scanned by UvsX/primer complexes and strand exchange is facilitated at homologous sequences. The resulting structures are stabilized by GP 32 interacting with the displaced template strand. UvsX disassembly leaves the end of the primer accessible to Bsu polymerase and primer extension subsequently. Exponential amplification is accomplished by the circulation of this process.

Table S1 Sequences of RPA primers used in this study.

Gene	Primer Name	Sequence (5'-3')	Primer Length (bp)	Optimal Concentration (μM)
CP4EPSPS	F I	ATTAACAACATGGCACAAGGGATACAAACC	30	0.36
	R I	CCACTGATGCTGAAATCCTAAAGGAACAAAAC	32	0.36
	F II	TCCCAATTCCAATTTCCATAAACCCCAAGT	30	0.36
	R II	AGGCTGTAGCCACTGATGCTGAAATCCTA	29	0.36
	F III	AACAACATGGCACAAGGGATACAAACC	27	0.36
	R III	CCACTGATGCTGAAATCCTAAAGGAAC	27	0.36
Lectin	F IV	TTTCCCCACGCCAACAAAACCTTATCTTCCAA	32	0.36
	R IV	GCTACCTACCTTGCCTGTTTCACTGTCCCA	30	0.36
	F V	GATGCTGCTATCTCACCCTCCG	22	0.52
	R V	GCTACCTTGCCTGTTTCACTGTC	23	0.48
	F VI	CCCACGCCAACAAAACCTTATCTTCC	26	0.36
	R VI	GCTACCTTGCCTGTTTCACTGTCCC	25	0.44

Figure S2

a)



b)

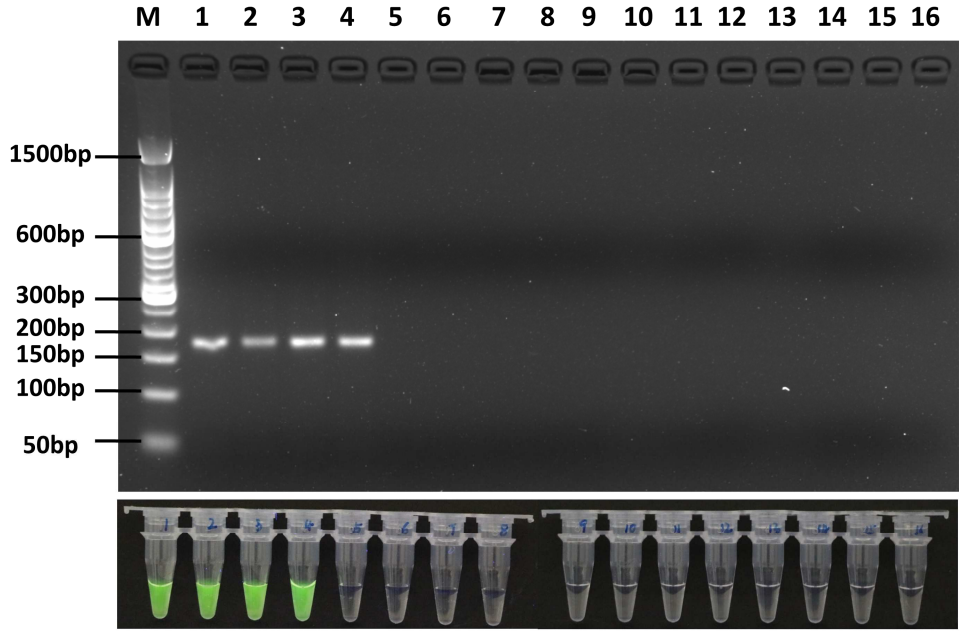


Figure S2 The images of gel electrophoresis and visual detection results of RPA assay for the determination of *CP4EPSPS* gene (a) and *lectin* gene (b). The tested samples were respectively GTS 40-3-2 soybean (lane 1, tube 1); DP 305423 soybean (lane 2,

tube 2), DP 356043 soybean (lane 3, tube 3), non-transgenic soybean (lane 4, tube 4); LL 601 rice (lane 5, tube 5), TT51-1 rice (lane 6, tube 6), non-transgenic rice (lane 7, tube 7), MON 810 maize (lane 8, tube 8), non-transgenic maize (lane 9, tube 9), oilseed rape (lane 10, tube 10), potato (lane 11, tube 11), beet (lane 12, tube 12), peanut (lane 13, tube 13), sugar cane (lane 14, tube 14), radish (lane 15, tube 15) and pumpkin (lane 16, tube 16), respectively. M, 50 bp DNA ladder.

Figure S3

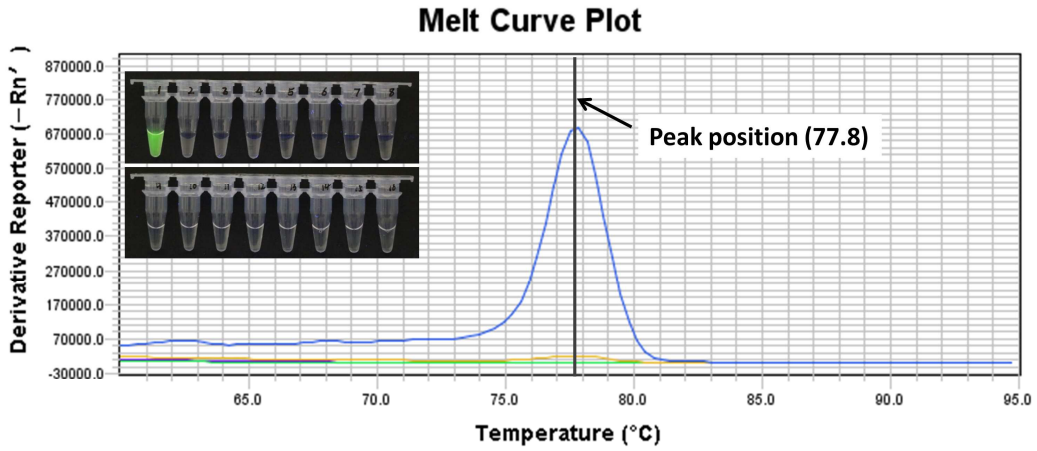


Figure S3 Melt curves of PCR assay for the detection of *CP4EPSPS* gene. The insert image shows visual detection results of SYBR Green I after PCR amplification. The 16 tested samples were GTS 40-3-2 soybean (blue curve, tube 1); DP 305423 soybean (tube 2), DP 356043 soybean (tube 3), non-transgenic soybean (tube 4), LL 601 rice (tube 5), TT51-1 rice (tube 6), non-transgenic rice (tube 7), MON 810 maize (tube 8), non-transgenic maize (tube 9), oilseed rape (tube 10), potato (tube 11), beet (tube 12), peanut (tube 13), sugar cane (tube 14), radish (tube 15) and pumpkin (tube 16) (overlapped horizon lines), respectively.

Figure S4

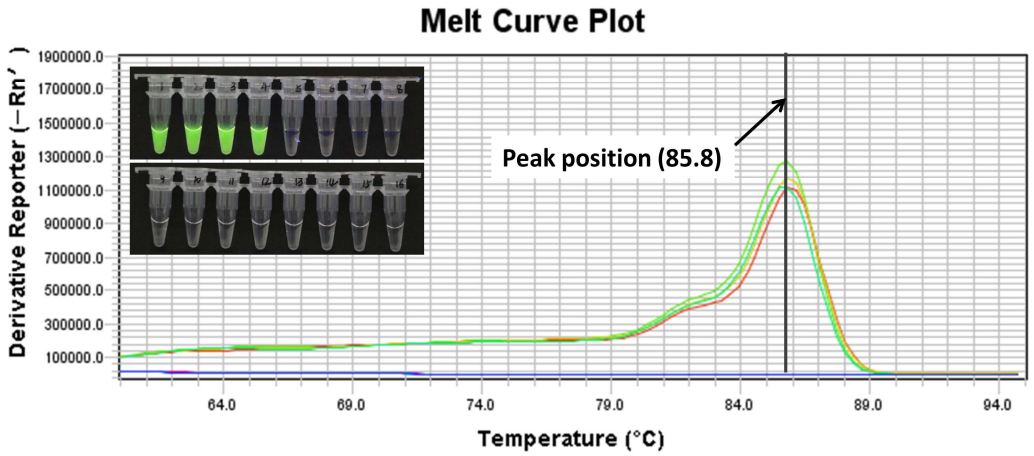


Figure S4 Melt curves of PCR assay for the detection of *lectin* gene. The insert image shows visual detection results based on SYBR Green I after PCR amplification. The 16 tested samples were GTS 40-3-2 soybean (light green curve, tube 1); DP 305423 soybean (dark green curve, tube 2), DP 356043 soybean (brown curve, tube 3), non-transgenic soybean (red curve, tube 4); LL 601 rice (tube 5), TT51-1 rice (tube 6), non-transgenic rice (tube 7), MON 810 maize (tube 8), non-transgenic maize (tube 9), oilseed rape (tube 10), potato (tube 11), beet (tube 12), peanut (tube 13), sugar cane (tube 14), radish (tube 15) and pumpkin (tube 16) (overlapped horizon lines), respectively.

Figure S5

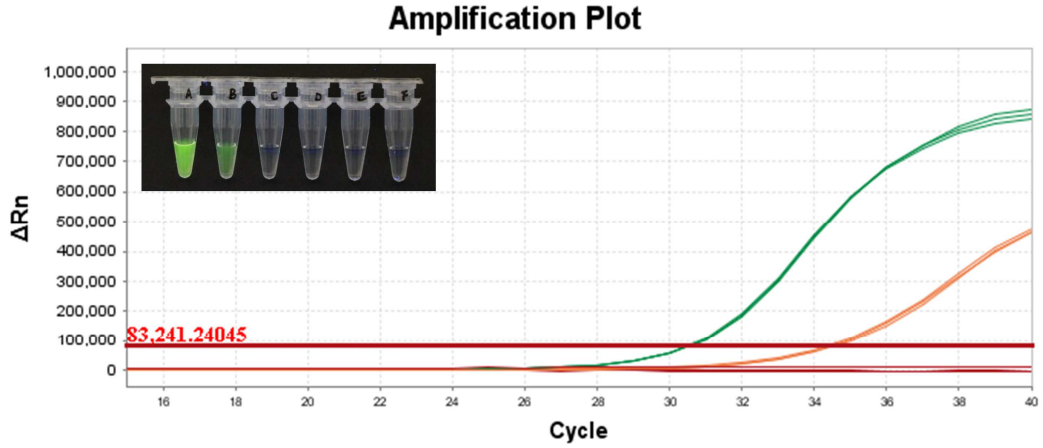


Figure S5 Amplification curves of real time PCR assay for the detection of *CP4EPSPS* gene. The initial DNA templates in each reaction were respectively 10^4 copies (green curves); 10^3copies (brown curves); 10^2, 10, 1, 0 copies (the horizontal crimson lines). Inset shows fluorescent detection results observed by naked eye after PCR amplification. From left to right, the initial DNA templates in each tube were 10^4, 10^3, 10^2, 10, 1, 0 copies, respectively.

Figure S6

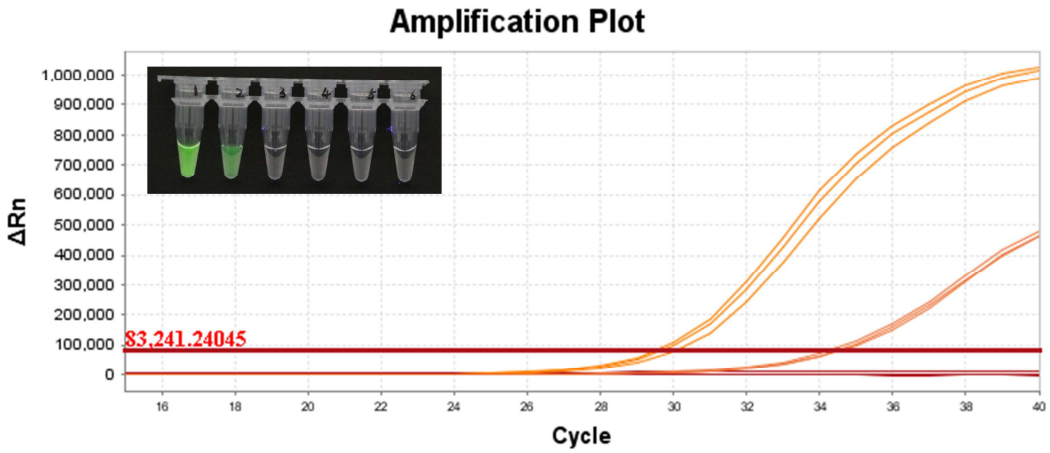
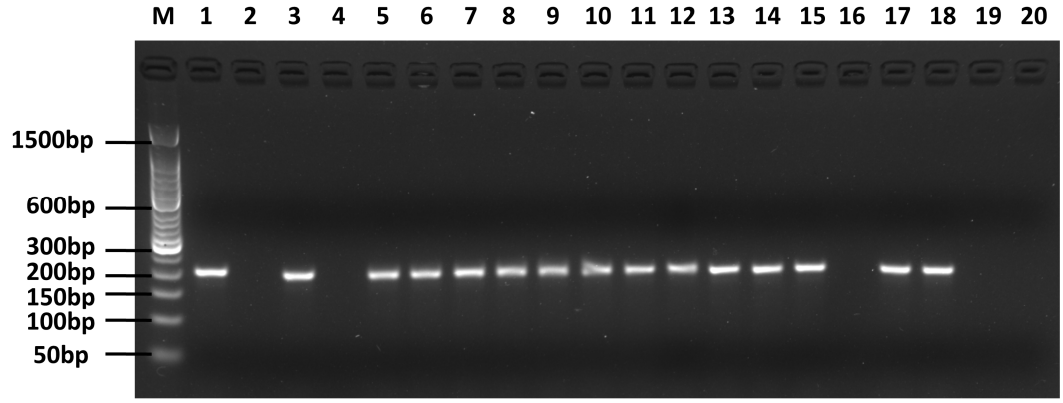


Figure S6 Amplification curves of real time PCR assay for the detection of *lectin* gene. The initial DNA templates in each reaction were respectively 10^4 copies (brownish yellow curves); 10^3 copies (brownish red curves); 10^2, 10, 1, 0 copies (the horizontal crimson lines). Inset shows visual detection results after PCR amplification. From left to right, the initial DNA templates in each tube were 10^4, 10^3, 10^2, 10, 1, 0 copies, respectively.

Figure S7

a)



b)

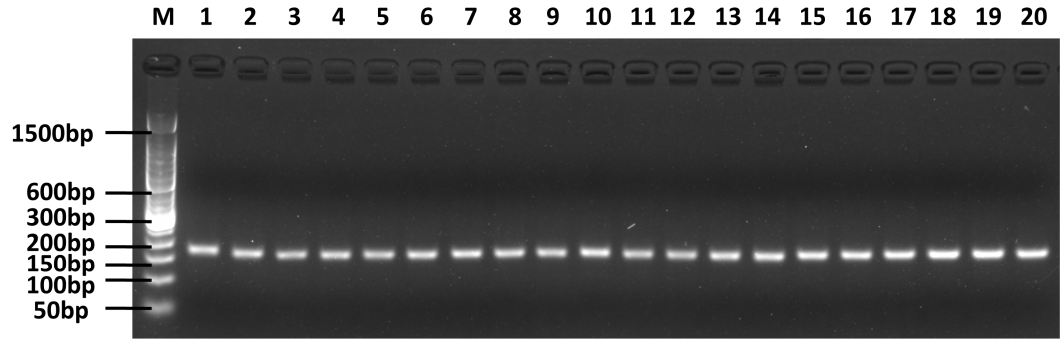


Figure S7 The gel electrophoresis image of PCR assay for the detection of *CP4EPSPS* gene (a) and *lectin* gene (b). All samples displayed positive results for *lectin* gene amplification, ensured the reliability of detection results. Based on this, 15 of 20 samples in total showed positive results for *CP4EPSPS* gene detection (except for samples of 2, 4, 16, 19, 20), and they were screened as GTS 40-3-2 soybean samples.