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5	Electronic Supplementary Information for
6	An instant, visual and instrument-free method for on-site screening of GTS
7	40-3-2 soybean based on body-heat triggered recombinase polymerase
8	amplification
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10	RuiWang, <sup>a</sup> Fang Zhang, <sup>b</sup> Liu Wang, <sup>a</sup> Wenjuan Qian, <sup>a</sup> Cheng Qian, <sup>a</sup> Jian Wu, <sup>a*</sup> and Yibin Ying <sup>a</sup>
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12	<sup>a</sup> College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China
13	<sup>b</sup> College of Biological Science and Engineering, Fuzhou University, Fuzhou, 350108, China
14 15	* (I Wu) E-mail: wujjan69@zju edu en
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17	Figure S1 The schematic of RPA amplification.
18	Table S1 Sequences of RPA primers used in this study.
19	Figure S2 Specificity evaluation of selected primers for the detection of CP4EPSPS
20	gene and <i>lectin</i> gene through RPA amplification.
21	Figure S3 and S4 Specificity evaluation of selected primers for the detection of
22	CP4EPSPS gene and lectin gene by PCR assay.
23	Figure S5 and S6 Sensitivity evaluation of selected primers for the detection of
24	CP4EPSPS gene and lectin gene by PCR assay.
25	Figure S7 PCR amplification as control for the detection of 20 real samples.
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#### 28 Materials and reagents

29 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, 30 China); transgenic soybean DP 305423 and DP 356043 (purchased from DuPont 31 32 Pioneer Hi-bred International, Inc., MA, USA); transgenic rice LL 601 (purchased 33 from Monsanto Co., MO, USA); transgenic rice TT51-1 (obtained from Huazhong Agricultural University, Wuhan, China); transgenic maize MON 810 (purchased from 34 35 Monsanto Co. MO, USA); non-transgenic soybean, maize and rice (purchased from 36 local market, Hangzhou, China); oilseed rape, potato, beet, peanut, sugar cane, radish, pumpkin (purchased from the local market, Hangzhou, China). 37 38 Reagents of sodium hydroxide (NaOH) and agarose were both purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All primers were 39 40 synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). TIANamp plant DNA kit 41 was purchased from Tiangen Biotech Co., Ltd. (Beijing, China) for standard DNA

46 was purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

TwistDx Ltd. (Hertfordshire, AL UK) for RPA amplification.

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extraction. TaKaRa Taq<sup>™</sup> Hot Start amplification kit was purchased from Takara Bio

Inc. (Dalian, China) for PCR amplification. TwistAmp<sup>®</sup> Basic kit was purchased from

purchased from Thermo Fisher Scientific Inc. (Waltham, MA USA). SYBR Green I

SYTO 9 was



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.

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GenePrimerSequence (5'-3')LengthConcentrationName(bp)(µM)F1ATTAACAACATGGCACAAGGGATACAAACC300.36R1CCACTGATGCTGAAATCCTAAAGGAACAAAAC320.36R11CCACTGATGCTGAAATCCTAAAGGAACCAAAGC300.36R11AGGCTGTAGCCACTGATGCTGAAATCCTAA290.36F111AACAACATGGCACAAGGGATACAAACC270.36R111CCACTGATGCTGAAATCCTAAAGGAAC270.36R111CCACTGATGCTGAAATCCTAAAGGAAC270.36R111CCACTGATGCTGCAACAAAACCTTATCTTCCAA320.36R111CCACTGATGCTGCTACTTGCCTGTTCCACTGTCCCA300.36R111CCACTGCCACGCCAACAAAACCTTATCTTCCAA320.36R111CCACTGCTGCTGTTTCACTGTCCC300.36R111CCACTGCCCACCACCAACAAAACCTTATCTTCC200.36R111CCACTGCCCACCACCAACAAAACCTTATCTTCC230.48F1VGCTACCTTGCCTGTTTCACTGTCC250.446465666767					Primer	Optimal
Name         (bp)         (μM)           F1         ATTAACAACATGGCACAAGGGATACAAACC         30         0.36           R1         CCACTGATGCTGAAATCCTAAAGGAACAAAAC         32         0.36           CP4EPSPS         F11         TCCCAATTCCAATTTCCATAAAGCACAAAAC         32         0.36           R1         AGGCTGTAGCCACTGATGCTGAAATCCTAA         29         0.36           F11         TCCCAATGGCACAAGGGATACAAACC         27         0.36           R11         AAGACATGGCACAAGGGATACAAACC         27         0.36           R11         CCACTGATGCTGAAATCCTAAAGGAAC         22         0.36           R11         CCACTGATGCTGACACAAGGGATACAAACC         27         0.36           R11         CCACTGATGCTGATGCTGAAATCCTAAAGGAAC         27         0.36           R11         CCACTGATGCTGATGCTGATACTAATGGAAC         27         0.36           R11         CCACTGATGCTGATGCTGATACTAATGGAAC         27         0.36           R11         CCACTGATGCTGCTGCTGTTTCACTGTCTCCAA         30         0.35           R11         CCACTGATGCTGCTGTTTCACTGTCTCCC         30         0.36           R1V         GCTACCTTGCCTGTTTCACTGTCCC         23         0.48           F V1         CCCACGCCAACAAAACCTTATCTTACTGTCC         25         0.44		Gene	Primer	Sequence (5'-3')	Length	Concentration
F1ATTAACAACATGGCACAAGGGATACAAACC300.36R1CCACTGATGCTGAAATCCTAAAGGAACAAAAC320.36R1TCCCAATTCCAATTTCCATAAAGCACAAGT300.36R11AGGCTGTAGCCACTGATGCTGAAATCCTA290.36F111AACAACATGGCACAAGGGATACAAACC270.36R11CCACTGATGCTGAAATCCTAAAGGAAC270.36R11CCACTGATGCTGAAATCCTAAAGGAAC270.36R11CCACTGATGCTGAAATCCTAAAGGAAC270.36R11CCACTGATGCTGATGCTGAAATCCTAAAGGAAC270.36R11CCACTGATGCTGCTGTTTCACTGTCCCA300.36R11CCACTGCCACGCCAACAAAACCTTATCTTCCAA320.36R11CCACTGCCTACCTTGCCTGTTTCACTGTCC300.36R11CCACCGCCAACAAAACCTTATCTTCC230.48F V1CCCCACGCCAACAAAACCTTATCTTCC260.36R V1GCTACCTTGCCTGTTTCACTGTCCC250.4464656667			Name		(bp)	(µM)
R1         CCACTGATGCTGAAATCCTAAAGGAACAAAAC         32         0.36           CP4EPSPS         F11         TCCCAATTCCAATTTCCATAAACCCCAAGT         30         0.36           R11         AGGCTGTAGCCACTGATGCTGAAATCCTA         29         0.36           F11         AACAACATGGCACAGGGGATACAAACC         27         0.36           R11         CCACTGATGCTGAAATCCTAAAGGAAC         27         0.36           R11         CCACTGATGCTGAAATCCTAAAGGAAC         27         0.36           R11         CCACTGATGCTGCAACAAAAGGGAAC         27         0.36           R11         CCACTGATGCTGCTGAAATCCTAAAGGAAC         27         0.36           R11         CCACTGATGCTGCTGCTGAAATCCTAAAGGAAC         27         0.36           R11         CCACTGATGCTGCTGCTGAAACCTAATCTTACCTGA         32         0.36           R11         GCTACCTACCTTGCCTGTTTCACTGTCCC         30         0.36           R1V         GCTACCTTGCCTGTTTCACTGTCC         23         0.48           FV1         CCCACGCCAACAAAAACCTTATCTTCC         26         0.36           RV1         GCTACCTTGCCTGTTTCACTGTCCC         25         0.44           64         65         66         67         5         5			FI	ATTAACAACATGGCACAAGGGATACAAACC	30	0.36
CP4EPSPS         F II         TCCCAATTCCAATTTCCATAAACCCCAAGT         30         0.36           R II         AGGCTGTAGCCACTGATGCTGAAATCCTA         29         0.36           F III         AACAACATGGCACAAGGGATACAAACC         27         0.36           R III         CCACTGATGCTGAAATCCTAAAGGAAC         27         0.36           R III         CCACTGATGCTGAAATCCTAAAGGAAC         27         0.36           F IV         TTTCCCCACGCCAACAAAAACCTTATCTTCCAA         32         0.36           R IV         GCTACCTACCTTGCCTGTTTCACTGTCCCA         30         0.36           I Eectin         F V         GATGCTGCTATCTTGCCTGTTTCACTGTCC         23         0.48           F V         GCTACCTTGCCTGTTTCACTGTCCC         25         0.44           64         65         66         5         5         5			RI	CCACTGATGCTGAAATCCTAAAGGAACAAAAC	32	0.36
R IIAGGCTGTAGCCACTGATGCTGAAATCCTA290.36F IIIAACAACATGGCACAAGGGATACAAACC270.36R IIICCACTGATGCTGAAATCCTAAAGGAAC270.36F IVTTTCCCCACGCCAACAAAAACCTTATCTTCCAA320.36R IVGCTACCTACCTTGCCTGTTTCACTGTCCCA300.36LectinF VGATGCTGCTATCTGCCTGTTTCACTGTC230.48F VICCCACGCCAACAAAACCTTATCTTCC260.36R VIGCTACCTTGCCTGTTTCACTGTCC250.4464656667		CP4EPSPS	FII	TCCCAATTCCAATTTCCATAAACCCCAAGT	30	0.36
FIII       AACAACATGGCACAAGGGATACAAACC       27       0.36         R III       CCACTGATGCTGAAATCCTAAAGGAAC       27       0.36         FIV       TTTCCCCACGCCAACAAAAACCTTATCTTCCAA       32       0.36         R IV       GCTACCTACCTTGCCTGTTTCACTGTCCCA       30       0.36         Ecctin       FV       GATGCTGCTATCTCACCCTCCG       22       0.52         R V       GCTACCTTGCCTGTTTCACTGTC       23       0.48         F VI       CCCACGCCAACAAAACCTTATCTTCC       26       0.36         R VI       GCTACCTTGCCTGTTTCACTGTCCC       25       0.44         64       65       66       67       67       67			R II	AGGCTGTAGCCACTGATGCTGAAATCCTA	29	0.36
R IIICCACTGATGCTGAAATCCTAAAGGAAC270.36F IVTTTCCCCACGCCAACAAAACCTTATCTTCCAA320.36R IVGCTACCTACCTTGCCTGTTTCACTGTCCCA300.36EectinF VGATGCTGCTATCTCACCCTCCG220.52R VGCTACCTTGCCTGTTTCACTGTC230.48F VICCCACGCCAACAAAACCTTATCTTCC260.36R VIGCTACCTTGCCTGTTTCACTGTCC250.44646566666766			F III	AACAACATGGCACAAGGGATACAAACC	27	0.36
FIVTTTCCCCACGCCAACAAAACCTTATCTTCCAA320.36R IVGCTACCTACCTTGCCTGTTTCACTGTCCCA300.36LectinFVGATGCTGCTATCTCACCCTCCG220.52R VGCTACCTTGCCTGTTTCACTGTC230.48F VICCCACGCCAACAAAACCTTATCTTCC260.36R VIGCTACCTTGCCTGTTTCACTGTCCC250.4464656667			R III	CCACTGATGCTGAAATCCTAAAGGAAC	27	0.36
R IVGCTACCTACCTTGCCTGTTTCACTGTCCCA300.36LectinFVGATGCTGCTATCTCACCCTCCG220.52R VGCTACCTTGCCTGTTTCACTGTC230.48F VICCCACGCCAACAAAACCTTATCTTCC260.36R VIGCTACCTTGCCTGTTTCACTGTCCC250.4464656667			F IV	TTTCCCCACGCCAACAAAACCTTATCTTCCAA	32	0.36
LectinFVGATGCTGCTATCTCACCCTCCG220.52RVGCTACCTTGCCTGTTTCACTGTC230.48F VICCCACGCCAACAAAACCTTATCTTCC260.36R VIGCTACCTTGCCTGTTTCACTGTCCC250.4464656667			R IV	GCTACCTACCTTGCCTGTTTCACTGTCCCA	30	0.36
R V       GCTACCTTGCCTGTTTCACTGTC       23       0.48         F VI       CCCACGCCAACAAAACCTTATCTTCC       26       0.36         R VI       GCTACCTTGCCTGTTTCACTGTCCC       25       0.44         64       65       66       67       67		Lectin	FV	GATGCTGCTATCTCACCCTCCG	22	0.52
F VI       CCCACGCCAACAAAACCTTATCTTCC       26       0.36         R VI       GCTACCTTGCCTGTTTCACTGTCCC       25       0.44         64			R V	GCTACCTTGCCTGTTTCACTGTC	23	0.48
R VI         GCTACCTTGCCTGTTTCACTGTCCC         25         0.44           64         65         66         67         66         67         66         67         66         67         66         67         66         67         <			F VI	CCCACGCCAACAAAACCTTATCTTCC	26	0.36
64 65 66 67			R VI	GCTACCTTGCCTGTTTCACTGTCCC	25	0.44
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**Table S1** Sequences of RPA primers used in this study.

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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,

88	tube 2), DP 356043 soybean (lane 3, tube 3), non-transgenic soybean (lane 4, tube 4);
89	LL 601 rice (lane 5, tube 5), TT51-1 rice (lane 6, tube 6), non-transgenic rice (lane 7,
90	tube 7), MON 810 maize (lane 8, tube 8), non-transgenic maize (lane 9, tube 9),
91	oilseed rape (lane 10, tube 10), potato (lane 11, tube 11), beet (lane 12, tube 12),
92	peanut (lane 13, tube 13), sugar cane (lane 14, tube 14), radish (lane 15, tube 15) and
93	pumpkin (lane 16, tube 16), respectively. M, 50 bp DNA ladder.
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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 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 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);  $10^3$ copies (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 Amplification curves of real time PCR assay for the detection of *lectin* gene. The initial DNA templates in each reaction were respectively 10<sup>4</sup> copies (brownish yellow curves); 10<sup>3</sup> copies (brownish red curves); 10<sup>2</sup>, 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<sup>4</sup>, 10<sup>3</sup>, 10<sup>2</sup>, 10, 1, 0 copies, respectively.

187 **a)** 



189 **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.