

Digital PCR-based Characterization of a g10evo-epsps Gene-Specific Matrix Reference Material for its Food and Feed Detection

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Table S1

Determination of seed moisture content of ZUTS-33 soybean.

Sample	Moisture content (%)					Mean (%)	SD	RSD (%)
	1	2	3	4	5			
ZUTS-33 soybean	7.05	7.47	7.36	6.94	7.56	7.28	0.003	3.69

Table S2

DNA quality extracted with different isolation kit.

Kit	OD ₂₆₀ /OD ₂₈₀	OD ₂₆₀ /OD ₂₃₀	DNA concentration /ng·μL ⁻¹	DNA content /μg
CTAB	1.87±0.01	2.39±0.01	56.9±1.0	5.69±0.10
SDS	1.96±0.01	1.15±0.01	100.9±3.0	10.09±0.30
Rapid isolation	1.08±0.06	1.18±0.01	152.2±1.2	15.22±0.12

Table S3

Homozygote test result with ddPCR.

No of the Sample	Sample	Copies/20 μ LWell	Ratio	Result
1	<i>lectin</i>	4540	0.96	homozygote
	<i>g10evo-epsps</i>	4340		
2	<i>lectin</i>	4260	0.98	homozygote
	<i>g10evo-epsps</i>	4180		
3	<i>lectin</i>	3800	1.01	homozygote
	<i>g10evo-epsps</i>	3840		
4	<i>lectin</i>	4200	1.01	homozygote
	<i>g10evo-epsps</i>	4260		
5	<i>lectin</i>	4220	1.02	homozygote
	<i>g10evo-epsps</i>	4320		
6	<i>lectin</i>	4040	1.00	homozygote
	<i>g10evo-epsps</i>	4060		
7	<i>lectin</i>	4340	0.93	homozygote
	<i>g10evo-epsps</i>	4040		
8	<i>lectin</i>	3740	0.97	homozygote
	<i>g10evo-epsps</i>	3618		
9	<i>lectin</i>	3580	0.96	homozygote
	<i>g10evo-epsps</i>	3440		
10	<i>lectin</i>	3220	0.96	homozygote
	<i>g10evo-epsps</i>	3080		
11	<i>lectin</i>	4080	0.94	homozygote
	<i>g10evo-epsps</i>	3820		
12	<i>lectin</i>	4260	1.05	homozygote
	<i>g10evo-epsps</i>	4460		
13	<i>lectin</i>	3900	0.90	homozygote
	<i>g10evo-epsps</i>	3500		
14	<i>lectin</i>	4100	1.00	homozygote
	<i>g10evo-epsps</i>	4100		
15	<i>lectin</i>	4340	1.00	homozygote
	<i>g10evo-epsps</i>	4340		
16	<i>lectin</i>	4600	0.91	homozygote
	<i>g10evo-epsps</i>	4180		
17	<i>lectin</i>	3840	1.01	homozygote
	<i>g10evo-epsps</i>	3880		
18	<i>lectin</i>	3420	1.09	homozygote
	<i>g10evo-epsps</i>	3740		
19	<i>lectin</i>	3500	0.91	homozygote
	<i>g10evo-epsps</i>	3200		

20	<i>lectin</i>	3600	0.88	homozygote
	<i>g10evo-epsps</i>	3180		

Table S4

Results of *g10evo-epsps* homogeneity test (exogenous/endogenous copy number ratio)

<i>g10evo-epsps</i>						
Vails	Experiments			Mean	SD	RSD
	1	2	3			
1	1.005	1.019	1.008	1.011	0.007	0.007
2	0.946	0.981	0.969	0.965	0.018	0.019
3	0.946	0.974	0.985	0.968	0.020	0.020
4	0.994	1.016	0.988	0.999	0.015	0.015
5	0.978	0.964	0.974	0.972	0.007	0.007
6	1.026	0.997	1.002	1.008	0.015	0.015
7	1.018	0.966	1.000	0.995	0.026	0.026
8	0.954	0.955	0.998	0.969	0.025	0.026
9	0.955	0.954	1.021	0.977	0.038	0.039
10	0.994	0.992	0.963	0.983	0.017	0.018
11	0.978	1.000	0.948	0.975	0.026	0.027
12	0.978	0.960	0.974	0.971	0.010	0.010
	Total Mean				0.983	
	Total SD				0.016	
	Total RSD (%)				0.017	

Table S5

Results of certified value in eight laboratories.

Lab	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Mean	SD	RSD
1	0.98	0.97	0.96	0.97	0.98	0.96	0.97	0.96	0.970	0.01	0.99%
2	1.00	1.01	0.99	1.01	1.02	0.98	1.01	0.97	1.000	0.02	1.72%
3	0.99	1.01	1.02	1.01	1.02	1.00	1.02	1.00	1.009	0.01	1.07%
4	0.95	0.95	0.97	0.99	0.97	0.97	0.96	0.99	0.968	0.02	1.73%
5	0.96	1.00	0.97	0.99	0.93	0.98	0.98	0.94	0.968	0.02	2.47%
6	0.96	1.02	0.99	1.01	0.96	0.96	0.93	1.00	0.977	0.03	3.11%
7	0.95	0.98	0.92	0.98	0.96	0.96	0.95	0.99	0.960	0.02	2.26%
8	0.96	0.99	0.95	0.97	0.97	0.98	1.00	0.98	0.974	0.01	1.41%
Mean						0.978					
SD						0.018					
RSD						1.845%					

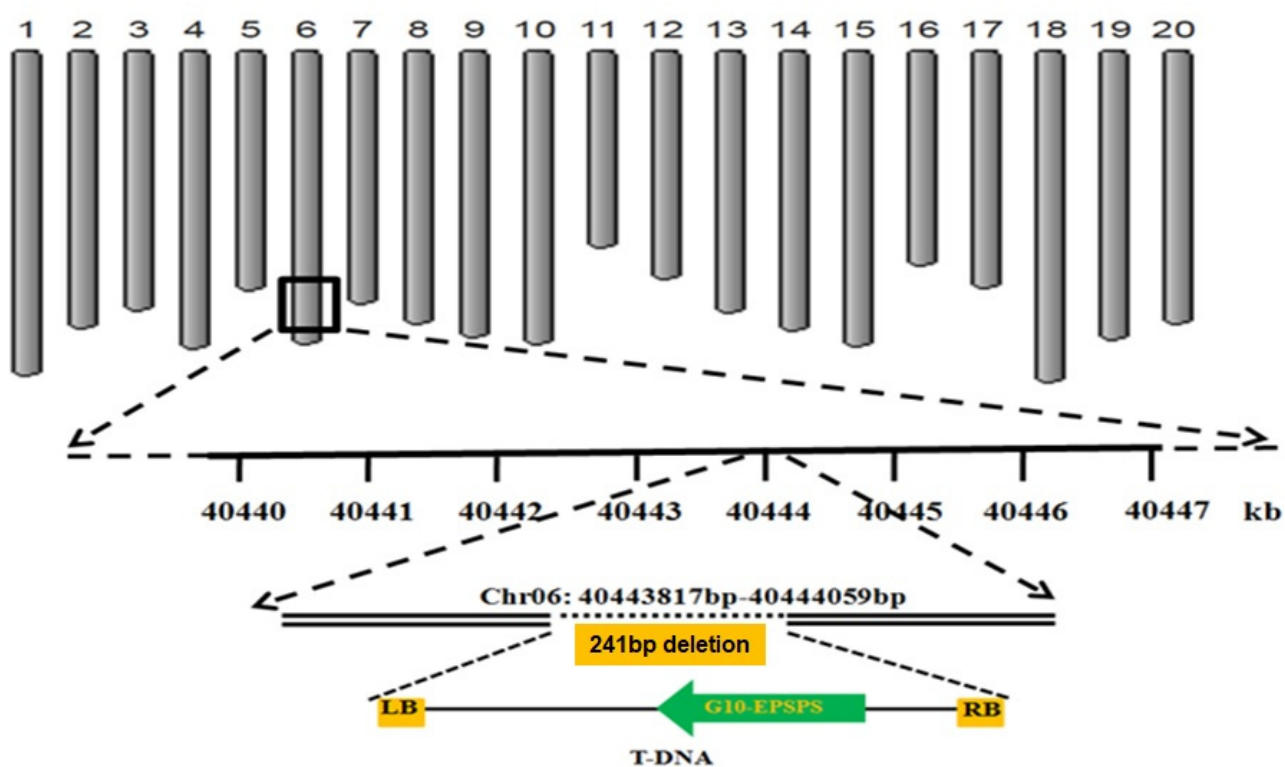


Figure S1. T-DNA structure and insertion site of the ZUTS-33 soybean

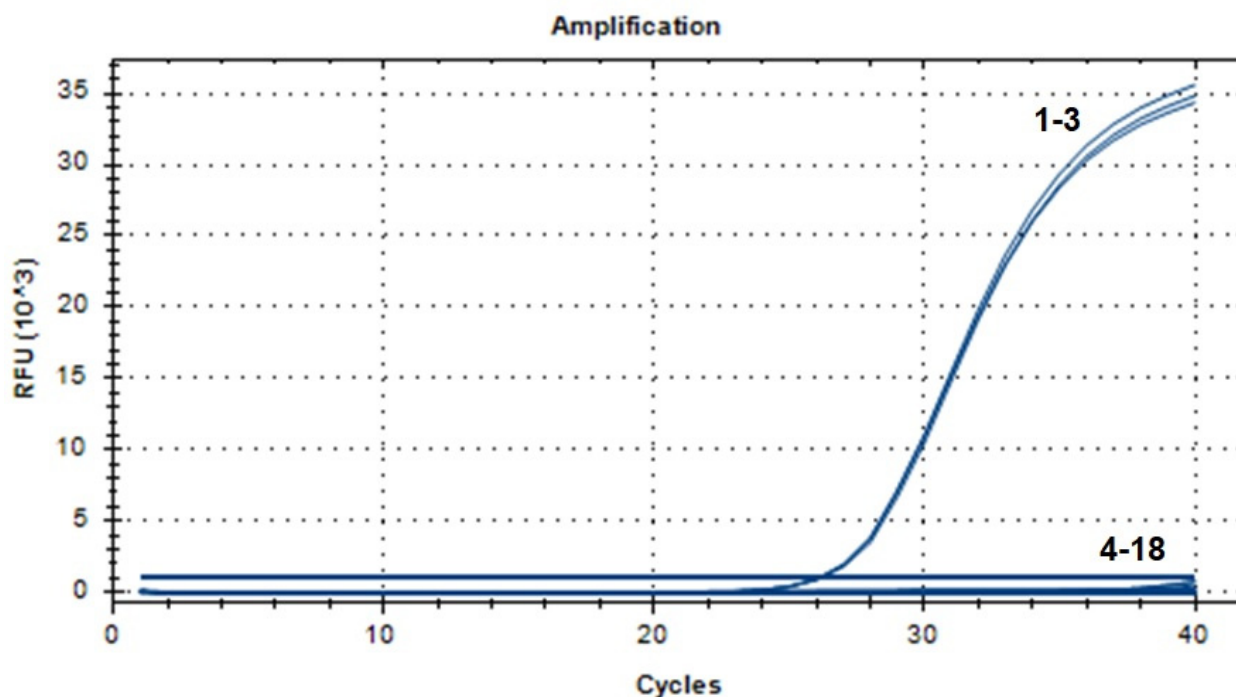


Figure S2. ZUTS-33 soybean event specific real time PCR amplification curve.

Line 1-3, three duplicates of GM soybean containing 0.5 ng; Line 4-6, three duplicates of mixed GM maize (Bt11, Bt176, MON810, MON863, GA21, NK603, T25, TC1507, MON89034, MON88017, 59122, MIR604, 3272, MON87460, MIR162, DAS40278-9, Shuangkang 12-5, IE09S034, C0030.3.5, C0010.3.7, 4114, MON87427, 5307, the content of each sample is 1%); Line 7-9, three duplicates of mixed GM soybean (GTS40-3-2, MON89788, CV127, A5547-127, A2704-12, 305423, 356043, MON88302, 73496, MON87769, MON87705, FG72, DAS68416-4, the content of each sample is 1%); Line 10-12, three duplicates of mixed GM rice (TT51-1, Kefeng 6, Kemingdao, M12, Kefeng 8, Kefeng 2, G6H1, T1C-19, the content of each sample is 1%); Line 13-15, three duplicates of mixed GM cotton (MON531, MON1445, MON15985, LLCOTTON25, MON88913, GHB614, COT102, the content of each sample is 1%); Line 16-18, three duplicates of mixed GM canola (Ms1, Ms8, RF1, RF2, RF3, T45, oxy235, Topas19/2, MON88302, 73496, the content of each sample is 1%).