

Table 2. Yield of DNA extracted from reference materials (0, 0.1, 0.5, 1 and 5% GM soybean), and copy number measured for each gene using real-time PCR

GMO amount (%)	DNA quantity (ng)		Usage for PCR	RRS	Copy number		
	Extraction from Sample				LeI	ColEI	
0	907 ± 81 (9)	11.3 ± 1.0 (9)			22010 ± 2090 (9)	12533 ± 940 (7)	
0.1	1063 ± 112 (11)	13.3 ± 1.4 (11)	26 ± 6 (24)	25999 ± 2725 (10)	12623 ± 724 (6)		
0.5	1093 ± 101 (9)	13.7 ± 1.3 (9)	107 ± 24 (22)	27480 ± 3107 (11)	14013 ± 635 (5)		
1	1202 ± 92 (8)	15.0 ± 1.2 (8)	227 ± 42 (18)	29043 ± 2932 (10)	14682 ± 805 (5)		
5	1246 ± 233 (19)	15.6 ± 2.9 (19)	1102 ± 264 (24)	28530 ± 7546 (26)	13894 ± 710 (5)		

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 3. Copy number ratio of RRS to Le1 or CoIE1 in the DNA extracted from reference materials

GMO amount (%)	Copy number ratio	
	RRS/Le1	RRS/CoIE1
0		
0.1	0.0010 ± 0.0001 (14)	0.0020 ± 0.0006 (28)
0.5	0.0039 ± 0.0005 (13)	0.0076 ± 0.0015 (19)
1	0.0078 ± 0.0008 (10)	0.0156 ± 0.0033 (21)
5	0.0392 ± 0.0073 (19)	0.0792 ± 0.0180 (23)

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 4. Yield of DNA extracted from the raw and processed foods spiked with 5% GM soybean powder (final GMO amount 0.5%), and copy number measured for each gene using real-time PCR

Sample	DNA quantity (ng)			Usage for PCR			Copy number		
	Extraction from Sample						LeI	ColE1	
Raw food									
Sugar	57 ± 19 (33)	0.7 ± 0.2 (33)	49 ± 14 (14)	1344 ± 225 (17)	6593 ± 1938 (29)				
Wheat	862 ± 28 (3)	10.8 ± 0.4 (3)	97 ± 17 (17)	2875 ± 156 (5)	11020 ± 1121 (10)				
Corn	140 ± 11 (8)	1.8 ± 0.1 (8)	117 ± 9 (9)	2976 ± 260 (9)	11665 ± 261 (2)				
Processed food									
Chocolate	68 ± 11 (16)	0.8 ± 0.13 (16)	68 ± 14 (21)	2011 ± 290 (14)	12487 ± 556 (4)				
Cookie	144 ± 21 (15)	1.8 ± 0.26 (15)	87 ± 6 (7)	3139 ± 660 (21)	11951 ± 1166 (10)				
Popcorn	135 ± 18 (14)	1.7 ± 0.23 (14)	89 ± 22 (25)	3454 ± 323 (9)	12749 ± 502 (4)				

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 5. Copy number ratio of RRS to Le1 or Cole1 in the DNA extracted from the food samples spiked with 5% GM soybean powder (final GMO amount 0.5%), and GMO amount calculated by the copy number ratio

Sample	Copy number ratio		GMO amount (%) calculated by the copy number ratio	
	RRS/Le1	RRS/Cole1	RRS/Le1	RRS/Cole1
Raw food				
Sugar	0.0364 ± 0.0062 (17)	0.0076 ± 0.0012 (16)	4.65 ± 0.80 (17)	0.48 ± 0.08 (16)
Wheat	0.0336 ± 0.0049 (15)	0.0088 ± 0.0015 (17)	4.29 ± 0.62 (15)	0.56 ± 0.09 (17)
Corn	0.0394 ± 0.0037 (9)	0.0100 ± 0.0008 (8)	5.04 ± 0.47 (9)	0.63 ± 0.05 (8)
Processed food				
Chocolate	0.0337 ± 0.0027 (8)	0.0055 ± 0.0012 (22)	4.30 ± 0.35 (8)	0.35 ± 0.08 (22)
Cookie	0.0284 ± 0.0061 (22)	0.0073 ± 0.0010 (13)	3.63 ± 0.78 (22)	0.46 ± 0.06 (13)
Popcorn	0.0257 ± 0.0058 (23)	0.0069 ± 0.0016 (23)	3.28 ± 0.74 (23)	0.44 ± 0.10 (23)

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 6. Copy number of RRS and ColE1 and RRS/ColE1 ratio measured by duplex real-time PCR in the DNA extracted from reference materials

GMO amount (%)	DNA quantity (ng)		Usage for PCR		Copy number		Copy number ratio
	Extraction from Sample				RRS	ColE1	RRS/ColE1
0	907 ± 81 (9)	11.3 ± 1.0 (9)			160 ± 37 (23)	64149 ± 3859 (6)	0.0023 ± 0.0005 (22)
0.1	1063 ± 112 (11)	13.3 ± 1.4 (11)			678 ± 104 (15)	69474 ± 7684 (11)	0.0089 ± 0.0015 (17)
0.5	1093 ± 101 (9)	13.7 ± 1.3 (9)			1457 ± 400 (27)	76436 ± 7288 (10)	0.0197 ± 0.0042 (21)
1	1202 ± 92 (8)	15.0 ± 1.2 (8)			7266 ± 423 (6)	73434 ± 7635 (10)	0.1022 ± 0.0073 (7)
5	1246 ± 233 (19)	15.6 ± 2.9 (19)				71160 ± 1897 (3)	

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 7. Copy number of RRS and CoIE1 and RRS/CoIE1 ratio measured by duplex real-time PCR and GMO amount calculated by RRS/CoIE1 ratio in the DNA extracted from the raw and processed foods spiked with 5%GM soybean powder (final GMO amount 0.5%)

Sample	DNA quantity (ng)		Copy number		Copy number ratio		GMO amount (%) calculated by RRS/CoIE1 ratio
	Extraction from Sample	Usage for PCR	RRS	CoIE1	RRS/CoIE1		
Raw food							
Sugar	57 ± 19 (33)	0.7 ± 0.2 (33)	283 ± 98 (35)	36502 ± 8974 (25)	0.0077 ± 0.0014 (18)	0.40 ± 0.07 (16)	
Wheat	862 ± 28 (3)	10.8 ± 0.4 (3)	692 ± 139 (20)	58565 ± 10570 (18)	0.0118 ± 0.0011 (9)	0.60 ± 0.05 (9)	
Corn	140 ± 11 (8)	1.8 ± 0.1 (8)	734 ± 243 (33)	65396 ± 1566 (2)	0.0112 ± 0.0036 (32)	0.57 ± 0.17 (30)	
Processed food							
Chocolate	68 ± 11 (16)	0.8 ± 0.13 (16)	448 ± 103 (23)	70552 ± 4104 (6)	0.0064 ± 0.0016 (25)	0.34 ± 0.08 (23)	
Cookie	144 ± 21 (15)	1.8 ± 0.26 (15)	683 ± 199 (29)	70612 ± 7073 (10)	0.0096 ± 0.0021 (22)	0.49 ± 0.10 (21)	
Popcorn	135 ± 18 (14)	1.7 ± 0.23 (14)	757 ± 143 (19)	86798 ± 5161 (6)	0.0087 ± 0.0014 (16)	0.45 ± 0.07 (15)	

Data represent mean ± SD (n=4). Numbers in parentheses indicate %CV values.

Table 8. Effect of capture oligos on Ct values and copy numbers measured for Le1 gene using PrimeSurface treated tube and real-time PCR from 100 ng of soybean DNA

Capture oligo	Ct value	Copy number
Upstream-s	34.2 ± 0.5	71 ± 23
Upstream-a	36.4 ± 0.5	15 ± 4
Le1n02-5'-s	33.9 ± 0.3	83 ± 14
Le1n02-5'-a	39.0 ± 0.4	3 ± 1
Le1-Taq-s	34.2 ± 0.2	69 ± 11
Le1-Taq-a	36.7 ± 1.0	13 ± 6
Le1n02-3'-s	36.8 ± 0.4	12 ± 3
Le1n02-3'-a	33.5 ± 0.2	110 ± 16
Downstream-s	36.6 ± 0.4	13 ± 4
Downstream-a	34.0 ± 0.2	76 ± 10
SSIIb3-3'	ND	ND
None	ND	ND

Data represent mean ± SD (n=4).

Table 9. Effect of capture oligo length on Ct values and copy numbers measured for Le1 gene using PrimeSurface treated tube and real-time PCR from 100 ng of soybean DNA

Concentration	Ct value	Copy number
Le1n02-3'-a (-8 mer)	36.8 ± 0.5	14 ± 4
Le1n02-3'-a	33.1 ± 0.1	157 ± 12
Le1n02-3'-a (+8 mer)	35.6 ± 0.5	32 ± 10

Data represent mean ± SD (n=4).

Table 10. Effect of capture oligo concentration on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR from 100 ng of soybean DNA

Concentration	Ct value	Copy number
0.1 uM	39.1 ± 1.0	2 ± 1
1 uM	35.9 ± 0.5	12 ± 4
10 uM	32.8 ± 0.4	101 ± 28

Data represent mean ± SD (n=4).

Table 11. Effect of incubation temperature on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR from 100 ng of soybean DNA

Temperature	Ct value	Copy number
25°C	35.1 ± 0.2	40 ± 5
40°C	33.5 ± 0.4	115 ± 35
50°C	32.9 ± 0.4	169 ± 43
60°C	33.6 ± 0.3	104 ± 22

Data represent mean ± SD (n=4).

Table 12. Effect of incubation time on Ct values and copy numbers measured for Le1 gene using LeIn02-3'-a attached tube and real-time PCR from 100 ng of soybean DNA

Time	Ct value	Copy number
15 min	33.7 ± 0.5	111 ± 35
30 min	33.6 ± 0.2	119 ± 16
60 min	33.2 ± 0.2	151 ± 15
120 min	32.9 ± 0.3	185 ± 33

Data represent mean ± SD (n=4).

Table 13. Effect of sample matrices on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR from 100 ng of soybean DNA

Sample matrix	Ct value	Copy number
Binding buffer	33.4 ± 0.3	74 ± 18
Corn extract	33.3 ± 0.7	85 ± 35
Wheat extract	32.9 ± 0.4	106 ± 25
Data represent mean ± SD (n=4).		

Table 14. Dependency of soybean DNA amount on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR

Amount	Ct value	Copy number
10 ng	35.8 ± 0.4	17 ± 5
100 ng	32.3 ± 0.2	174 ± 28
1000 ng	29.0 ± 0.4	1677 ± 493

Data represent mean ± SD (n=4).

Table 15. Dependency of soybean concentration on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR

Concentration	Ct value	Copy number
0.1%	38.0 ± 0.5	5 ± 1
1%	35.7 ± 0.2	22 ± 3
10%	32.1 ± 0.2	285 ± 51
100%	28.7 ± 0.1	3089 ± 206

Data represent mean ± SD (n=4).

Table 16. Effect of heat treatment on Ct values and copy numbers measured for Le1 gene using Le1n02-3'-a attached tube and real-time PCR from soybean sample

Time	Ct value	Copy number
0 min	28.3 ± 0.2	3578 ± 527
30 min	26.0 ± 0.1	15967 ± 800
60 min	27.0 ± 0.1	8011 ± 783

Data represent mean ± SD (n=4).

Table 17. Dependency of GM soybean concentration on Ct values and copy numbers measured for RRS gene using RRS-Tag-s attached tube and real-time PCR

Concentration	Ct value	Copy number
1%	39.6 ± 0.5	2 ± 2
10%	35.0 ± 0.3	47 ± 10
100%	30.6 ± 0.3	858 ± 143
Data represent mean ± SD (n=4).		

研究成果の刊行に関する一覧表

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