

表 1 リアルタイム PCR に用いたプライマー・プローブ

target	name	sequence (5'-3')	length (bp)
<i>SSIIB</i>	SSIIB3-5'	CCAATCCTTTGACATCTGCTCC	114
	SSIIB3-3'	GATCAGCTTTGGGTCCGGA	
	SSIIB-TaqV	VIC-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA	
	SSIIB-Taq	FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA	
P35S	P35S 1-5'	ATTGATGTGATATCTCCACTGACGT	101
	P35S 1-3'	CCTCTCCAAATGAAATGAACTTCCT	
	P35S-Taq	FAM-CCCCTATCCTTCGCAAGACCCCTTCCT-TAMRA	
tNOS	NOS ter 3-5'	GCATGTAATAATTAACATGTAATGCATGAC	108
	NOS ter 2-3'	CGCTATATTTTGTCTTCTATCGCGT	
	NOS-Taq	FAM-AGATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA	
55-1	55-1 primer1	CAGCCTTAGATGCTTCAAGAAAAGA	71
	55-1 primer2	TCCGCCCTCCATCCAGTCTATT	
	55-1 probe	FAM-TCTTCTAGCTTCCCGGCAACAAT-TAMRA	

表 2 リアルタイム PCR における検出限界 (LOD)

plasmid	pUC-P35S ^a			pUC-tNOS ^a			pUC-SSIIB		
	5	10	20	5	10	20	5	10	20
copy number	5	10	20	5	10	20	5	10	20
positive/total	15/21	21/21	21/21	15/21	19/21	21/21	12/21	19/21	20/21
positive rate	71.4	100.0	100.0	71.4	100.0	100.0	57.1	90.5	100.0
RSD (%)	4.3	3.2	1.3	3.5	1.8	2.9	3.0	3.1	2.3
LOD		✓				✓			✓

^a2 倍量の pUC-SSIIB を添加

表 3 抽出バッファー成分の PCR への影響評価

inhibitor	final concentration	pUC-P35S ^{a,b}		pUC-tNOS ^{a,b}		pUC-SSIIB ^a	
		<i>SSIIB</i>	P35S+tNOS	<i>SSIIB</i>	P35S+tNOS	<i>SSIIB</i>	P35S+tNOS
SDS	0.01%	4/4	4/4	4/4	4/4	4/4	0/4
	0.03% ^c	4/4	4/4	4/4	4/4	4/4	0/4
	0.3%	0/4	0/4	0/4	0/4	0/4	0/4
NaCl	10 mM	4/4	4/4	4/4	4/4	4/4	0/4
	40 mM ^c	4/4	4/4	4/4	4/4	4/4	0/4
	100 mM	0/4	0/4	0/4	0/4	0/4	0/4
EDTA	0.1 mM	4/4	4/4	4/4	4/4	4/4	0/4
	0.5 mM ^c	4/4	4/4	4/4	4/4	4/4	0/4
	5 mM	0/4	0/4	0/4	0/4	0/4	0/4
lysis buffer	0.1x	4/4	4/4	4/4	4/4	4/4	0/4

^a, 40 copies/ μ L. ^b, 2 倍量の pUC-SSIIB を添加. ^c, 抽出バッファーと同濃度.

表 4 ダルマピンを用いた DNA 抽出法の評価

GM maize variety	<i>SSIb</i>				P35S + tNOS			
	#1	#2	#3	#4	#1	#2	#3	#4
non-GM #1	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
non-GM #2	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
non-GM #3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
MON810	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
GA21	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Bt11	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
T25	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
NK603	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON863	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON89034	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MIR162	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON88017	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
DAS59122	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MIR604	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3272	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3

表 5 紙ヤスリを用いた DNA 抽出法の評価

GM maize variety	<i>SSIb</i>				P35S + tNOS			
	#1	#2	#3	#4	#1	#2	#3	#4
non-GM #1	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
non-GM #2	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
non-GM #3	3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/3
MON810	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
GA21	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Bt11	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
T25	3/3	3/3	3/3	3/3	3/3	2/3	3/3	3/3
NK603	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON863	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON89034	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MIR162	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MON88017	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
DAS59122	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MIR604	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
3272	1/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3

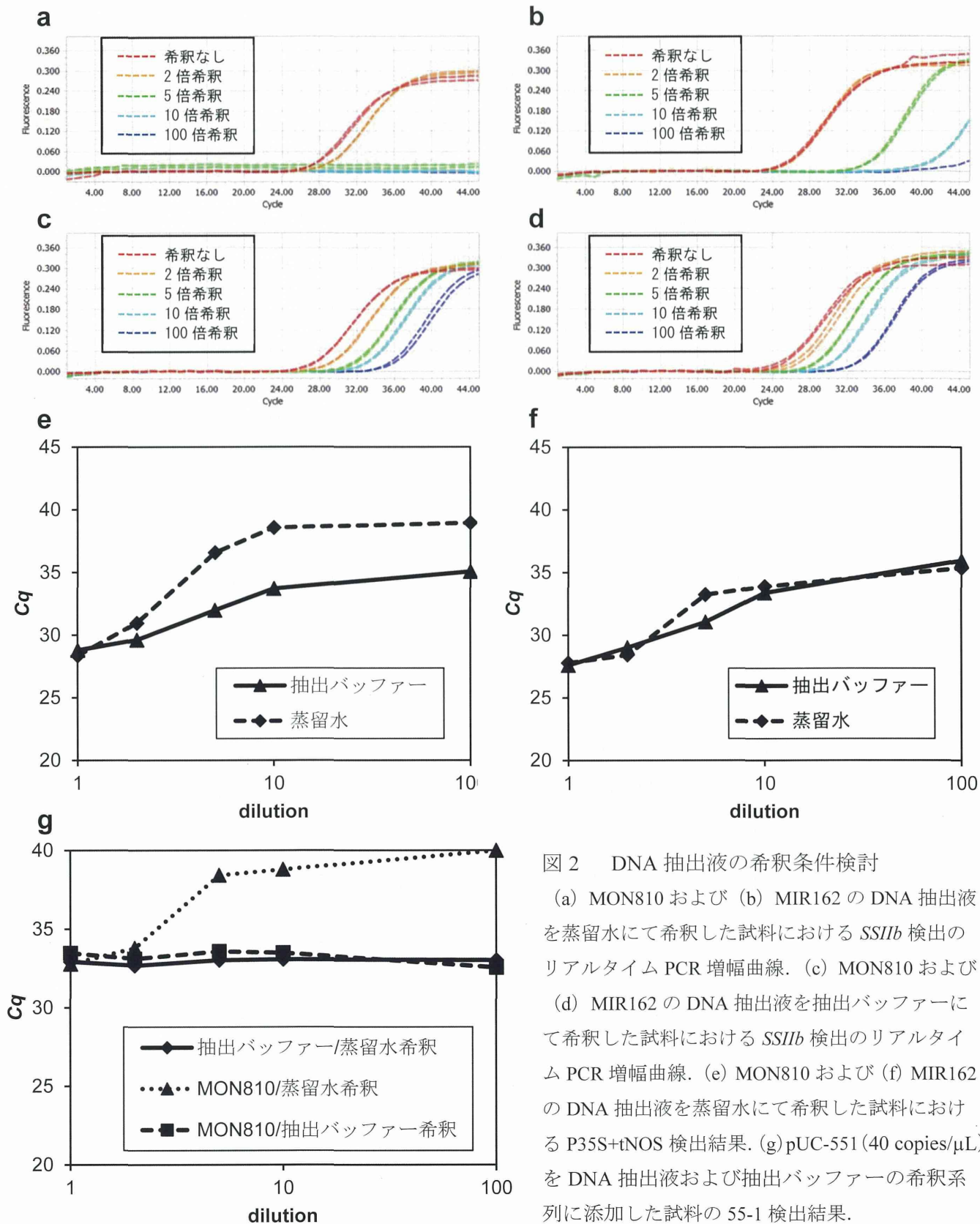


図2 DNA抽出液の希釈条件検討

(a) MON810および(b) MIR162のDNA抽出液を蒸留水にて希釈した試料における *SSI1b* 検出のリアルタイムPCR増幅曲線。(c) MON810および(d) MIR162のDNA抽出液を抽出バッファーにて希釈した試料における *SSI1b* 検出のリアルタイムPCR増幅曲線。(e) MON810および(f) MIR162のDNA抽出液を蒸留水にて希釈した試料における P35S+tNOS 検出結果。(g) pUC-551 (40 copies/ μ L)をDNA抽出液および抽出バッファーの希釈系列に添加した試料の 55-1 検出結果。

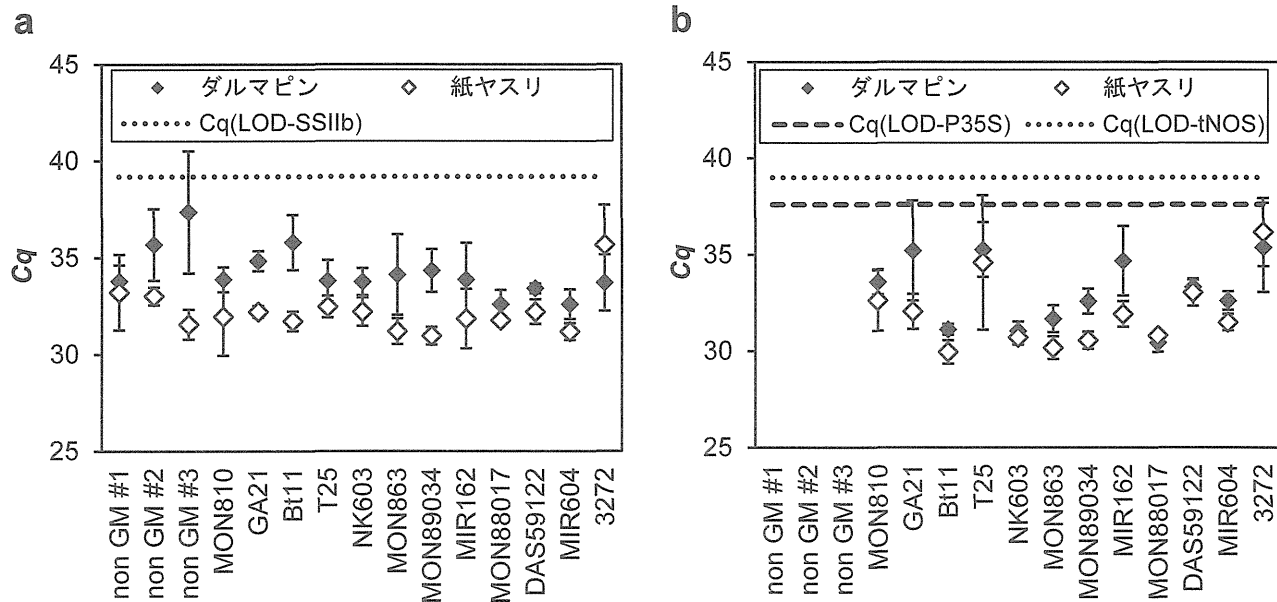


図3 DNA抽出法の検討における *SSIIb* (a) および P35S+tNOS (b) 検出に対する Cq 値

H26 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
今村知明		今村知明	【第2版】食品の安全とはなにかー食品安全の基礎知識と食品防衛	日本生活協同組合連合会出版部	東京	2015	p. 1-237
今村知明 他		今村知明	食品防衛の考え方とその進め方	日本食品衛生協会	東京	2015	p. 1-270

雑誌

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Mano, J., Hatano, S., Futo, S., Minegishi, Y., Ninomiya, K., Nakamura, K., Kondo, K., Teshima, R., Takabatake, R., Kitto, K	Development of direct real-time PCR system applicable to a wide range of food and agricultural products.	Food Hygiene and Safety Science	55	25-33	2014
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