

Supplementary material

LAMP-LFD Based on Isothermal Amplification of Multicopy Gene *ORF160b*: Applicability for Highly Sensitive Low-tech Screening of Allergenic Soybean (*Glycine max*) in Food

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Table S1. Specificity testing of two commercial protein-based LFD tests compared to *ORF160b* LAMP-LFD. Yellow soybean served as positive control (+: positive test line; -: negative test line).

Sample No.	Common Name	Species Name	Family	<i>ORF160b</i>		
				LAMP-LFD ³	LFD1	LFD2
1	peanut ^{1,2}	<i>Arachis hypogaea</i>	<i>Fabaceae</i>	-	-	-
2	sesame seed ¹	<i>Sesamum indicum</i>	<i>Pedaliaceae</i>	-	-	-
3	cow's milk ¹	<i>Bos taurus</i>	<i>Bovidae</i>	-	-	-
4	oat ¹	<i>Avena sativa</i>	<i>Poaceae</i>	-	-	-
5	barley ¹	<i>Hordeum vulgare</i>	<i>Poaceae</i>	-	-	-
6	rye ¹	<i>Secale cereale</i>	<i>Poaceae</i>	-	-	-
7	wheat ¹	<i>Triticum sp.</i>	<i>Poaceae</i>	-	-	-
8	brown lentil ²	<i>Lens culinaris</i>	<i>Fabaceae</i>	-	-	-
9	pea ²	<i>Pisum sativum</i>	<i>Fabaceae</i>	-	-	-
10	white mustard seed ¹	<i>Sinapis alba</i>	<i>Brassicaceae</i>	-	-	-
11	blue lupine ^{1,2}	<i>Lupinus angustifolius</i>	<i>Fabaceae</i>	-	-	-
12	yellow lupine ^{1,2}	<i>Lupinus luteus</i>	<i>Fabaceae</i>	-	-	-
13	white lupine ^{1,2}	<i>Lupinus albus</i>	<i>Fabaceae</i>	-	-	-
14	hazelnut ¹	<i>Corylus avellana</i>	<i>Betulaceae</i>	-	-	-
15	spelt ¹	<i>Triticum aestivum</i> subsp. <i>spelta</i>	<i>Poaceae</i>	-	-	-
16	squid ¹	<i>Loligo formosana</i>	<i>Loliginidae</i>	-	-	-
17	celeriac ¹	<i>Apium graveolens</i> var. <i>rapaceum</i>	<i>Apiaceae</i>	-	-	-
18	soybean ^{1,2}	<i>Glycine max</i>	<i>Fabaceae</i>	+	+	+

¹ require mandatory labeling as ingredient according to EU Directive 1169/2011; ² legume (*Fabaceae*);

³ previously published [18].

Table S2. Primers for *ORF160b* LAMP, *lectin* qPCR and eukaryotic *18S rRNA* qPCR used in this study.

Target Gene	primer name	sequence (5'→3')
<i>ORF160b</i> ¹	F3 ORF160b	CCGAGTCTGCTGCCGTAT
	B3 ORF160b	ATGAGATTGAGTTCCACGCA
	FIP ORF160b	GGGGTCAGTATTACGCCTCTGACAAAGAAAGAGAGTGACGATG
	biotin-FIP ORF160b	biotin-GGGGTCAGTATTACGCCTCTGACAAAGAAAGAGAGTGACGATG
	BIP ORF160b	TCTGATAGATAGTGGCAAACATTAGTTGCTGCTATTCCATCTATTCAT
	LoopF ORF160b	TTCTGATTCCGCTCATTGG
	FITC-LoopF ORF160b	FITC-TTCTGATTCCGCTCATTGG
	LoopB ORF160b	CAAGATATAGAAGACTATTAGCCCCG
<i>Lectin</i> ²	Lectin-F	TCCACCCCCATCCACATTT
	Lectin-R	GGCATAGAAGGTGAAGTTGAAGGA
	Lectin-probe	ROX-AACCGGTAGCGTTGCCAGCTTCG-BBQ-650
<i>18S rRNA</i> ³	TR03 (forward)	TCTGCCCTATCAACTTTCGATGGTA
	TR04 (reverse)	AATTGCGCGCCTGCTGCCTTCCTT

¹[18]; ²[20]; ³[21].

References

- [18] Allgöwer, S.M.; Hartmann, C.A.; Holzhauser, T. Sensitive detection of allergenic soybean (*Glycine max*) using multicopy gene loop-mediated isothermal amplification combined with lateral flow dipstick (LAMP-LFD). *Foods* **2020**, *9*(4):423, 2-19.
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- [21] Allmann, M.; Candrian, U.; Höfelein, C.; Lüthy, J. Polymerase chain reaction (PCR): a possible alternative to immunochemical methods assuring safety and quality of food, Detection of wheat contamination in non-wheat food products, *Z Lebensm Unters Forsch* **1993**, *196*(3), 248-251.



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