

Supporting Information

Article

Precise and rapid authenticity of functional food components using optimized TaqMan real-time quantitative PCR

Qiuyue Zheng^{1,†}, Xinying Yin^{1,†}, Aifu Yang², Ning Yu³, Ranran Xing³, Ying Chen^{3*}, Ruijie Deng⁴, Jijuan Cao^{1*}

¹ Key Laboratory of Biotechnology and Bioresources Utilization of Ministry of Education,

College of Life Science, Dalian Minzu University, Dalian 116600, China

² Technology Center of Dalian Customs District, Dalian 116001, China

³ Chinese Academy of Inspection and Quarantine, Beijing 322001, China

⁴ College of Biomass Science and Engineering, Healthy Food Evaluation Research Center, Sichuan University, Chengdu 610065, China

[†] These authors contributed equally to this work.

* Correspondence: chenyingcaiq@163.com (Y.C.); caojijuan@dlnu.edu.cn (J.C.)

A

		Forward primer				Reverse primer	
AF169231.1-Zizania_aquatic.txt	GA..GATATCCGGTGC...	GAGAGTCATGGGATGTT	416	AF169231.1-Zizania_aquatic.txt	GTAAGAGGGTT.CCCTTGACGCCGATGGCGCGTGGG...	492	
AF169232.1-Zizania_paluatris.txt	...-.......	g-	440	AF169232.1-Zizania_paluatris.txt	-----tg-t-----	516	
AF169233.1-Zizania_txana.txt	...-.......	g-	425	AF169233.1-Zizania_txana.txt	-----tg-t-----	501	
AF169234.1-Zizania_latifolia.txt	...-.......	g-	423	AF169234.1-Zizania_latifolia.txt	-----tg-t-----	499	
AF019792.1.txt	ac.-cc--c-cc-tcgggcgag-cg...-gacgc-g-	419	AF019792.1.txt	aa-gtcc--g.-tgcc-g--g-.c-----g-ca.c	488		
AF169230.1.txt	-cgccc--c-at---gggagc-gcgg...-gacgc-g-	434	AF169230.1.txt	-a-gtcc--g.-tgcc-g--aa..c-t----g-ca..c	507		
AF303400.1.txt	c.....ca..ccct-at...t-q- atcg-gacgc-g-	428	AF303400.1.txt	-a-gtcc--g.-tgcc-g--..t-cc-tt--aaa..c	496		
AY520821.1.txt	c.....c-cccttac...g-q- aaca-gacgc-g-	457	AY520821.1.txt	-a-gtcc--g..tccq-q-at---c-t----g-ca..c	524		
AY752478.1.txt	tgcggcccaagc-cct-ggacg-c-gccgc-gacgc-g-	561	AY752478.1.txt	tagggcta-agaat-attcgaaacgtct-t-aaa--aga	514		
DQ683012.1.txt	at...ggtt-q-at...-q-gtc--cct-ag-	434	DQ683012.1.txt	-a-gatt--g..tgcc-g--..t-cc-----gaca..c	497		
DQ981410.1.txt	c.....-accct-aa..c-q-g..-tcg-gatgc-gc	429	DQ981410.1.txt	-a-gatt--g..tgcc-g--..t-cc-----gaca..c	497		
GU359188.1.txt	c.....cccc-accc-aa..c-q-t-c-g...-acg--g-	449	GU359188.1.txt	-a-gtcc--g..tgcc-g--at--c-c-----g-ac..a	520		
HM347039.1.txt	ct.....-c-accc-gg..t-c-q-a..cg-q--g-	497	HM347039.1.txt	-a-gtcc--g..tgcc-g--..t-cc-tt--g-cac..a	566		
HQ329791.1.txt	cc.....-a-cacc-at...c-t-q-ag...-gacgc-gc	521	HQ329791.1.txt	-a-gat--g..tgcc-g--..t-cc-tt--g-cac..a	590		
JX576677.1.txt	c.....-c-a-cottg..g-tac-g...-acg--g-	394	JX576677.1.txt	-a-gtcc--g..tgcc-g-a..t-cc-tt--g-cac..c	463		
KC193780.1.txt	c.....-c-acctt..c-q-attg-gatgc-gc	424	KC193780.1.txt	-a-gatc--g..tgccag--..t-cc-----aaaaat	494		
KC90704.1.txt	--.a...-c-cccca-ggggc-d-c-qa...-gacgc-gc	468	KC90704.1.txt	-a-gtcc--g..-gce-g--ta-ggc-----g-ca..c	540		
KM036294.1.txt	ac...-cccc-c...-c-q-q...-g-cgg...-gacgc-g-	2213	KM036294.1.txt	-a-gtcc--g..tgcc-g--aa..c-t----g-ca..c	2281		
KX016112.1.txt	cc.gccc--ccccaaagggtcg-c-q-a...-gacgc-g-	558	KX016112.1.txt	-a-gacg--g..tgcc-g--aa..c-t----g-ca..c	626		
KY826234.1.txt	c.....cccc-cctt-ac..c-q-g-agc-gacgc-g-	2236	KY826234.1.txt	-a-gatc--g..tgcc-g--..t-cc-tt--g-cac..c	2304		
KY826235.1.txt	c.....cccc-ccctt-ac..c-q-g-agc-gacgc-g-	2236	KY826235.1.txt	-a-gatc--g..tgcc-g--..at-c-tt--g-cac..c	468		
MH762138.1.txt	c.....-c-acctt-g...-g-qag...-atg--g-	399	MH762138.1.txt	-a-gtcc--g..tgcc-g--..tttt-ta-tt-gtcac..a	509		
MK863204.1.txt	cc.....-c-accc...-g-t-c-g...-acg--g-	440	MK863204.1.txt	-a-gtcc--g..tgcc-g--at--c-c-----g-ac..a	561		
OM980632.1.txt	c.....-c-accc...-g-t-c-g...-acg--g-	490	OM980632.1.txt	-a-gtcc--g..tgcc-g--at--c-c-----g-ac..a	561		

B

		Forward primer				Probe	
XM_004507840.3.txt	GCGGCGCCGGTGACGGACAAGAAGAAAAAG	AGAAGGTTG	1433	XM_004507840.3.txt	AA[TACGAAGGCCGTTGGAAATGCTGGCTGTATAAG]GGAA	1553	
AB198917.1.txt	cat-a.ta-a--gt--ttgt-tgtcgc-c--gaccaa-ca	556	AB198917.1.txt	615		
AB257353.1.txt	att--ctttt-tgg-cg--agcttgc-tt-t-gca-agt	453	AB257353.1.txt	514		
AJ639943.1.txt	cat-a.ta-a--gt--ttgt-tgtcgc-c--gaccaa-ca	556	AJ639943.1.txt	615		
GO241316.1.txt	-t-a..-a-ta-gt.....	164	GO241316.1.txt	164		
KP338131.1.txt	cat-a.ta-a--gt--ttgt-tgtcgc-c--gaccaa-ca	588	KP338131.1.txt	665		
KP338509.1.txt	-gtt.gg-cc--ttctcc--tttgg-tattatt-agc-	697	KP338509.1.txt	773		
XM_004507840.3.txt	[TACGGTGGAAACACAACGGAGGTGTTGAAAGAAC	1473	XM_004507840.3.txt	CACCGTTCGCTGACGACA[TCCTATCTCCGACGCCACCG]GG	1593		
AB198917.1.txt	-g-c--ccct-ttga-tttt--cc-ct-gt-cc-ttt	595	AB198917.1.txt	615		
AB257353.1.txt	cattaa---tgctac-tgggtt-atcc--ccagt--tc-t	493	AB257353.1.txt	514		
AJ639943.1.txt	-g-c--ccct-ttga-tttt--cc-ct-gt-cc-ttt	595	AJ639943.1.txt	615		
GO241316.1.txt	164	GO241316.1.txt	164		
KP338131.1.txt	-g-c--ccct-ttga-tttt--cc-ct-gt-ccc-ttt	627	KP338131.1.txt	665		
KP338509.1.txt	a...tt-ttgcgt--t-tgca--aatc--tc-c-ttattc-	734	KP338509.1.txt	773		

Figure S1. Specific analysis of primer and probe. (A) Specificity comparison of primer and probe sequence for the ITS gene region developed using MEGA 4.0 for the specific qPCR detection of wild rice source component. (B) Specificity comparison of primer and probe sequences for the CIA-2 gene region developed for specific qPCR detection of chickpea source component using MEGA 4.0.

Table S1. Conditions of robustness test as an orthogonal design ^a

Factors	Combination								Control
	1	2	3	4	5	6	7	8	
qPCR instrument	A	A	A	A	B	B	B	B	B
qPCR reagent	X	X	Y	Y	X	X	Y	Y	X
Primer concentration (pmol/µL)	Unchanged	-20%	Unchanged	-20%	Unchanged	-20%	Unchanged	-20%	Unchanged
Probe concentration (pmol/µL)	Unchanged	-20%	-20%	Unchanged	-20%	Unchanged	Unchanged	-20%	Unchanged
Volume of reaction mixture (2×)	13.5 µL	13.5µL	11.5 µL	11.5µL	13.5 µL	13.5µL	11.5 µL	11.5µL	Unchanged
Annealing temperature	+1°C	- 1°C	+1°C	- 1°C	- 1°C	+1°C	- 1°C	+1°C	Unchanged

^a A : LightCycler® 480 II Real-Time Fluorescence PCR (Roche, Germany). B: QuantStudio6 Flex Real-Time Fluorescence PCR (ABI, USA). X: Premix Ex TaqTM Probe qPCR, RR390 A, TAKARA, China. Y: GoTaq qPCR Master Mix, A6101, Promega, USA.

Table S2. qPCR specific analysis results of quinoa, coix seed, wild rice and chickpea

Samples		Average Ct value				
Common name	Latin name	wild rice system	quinoa system	coix seed system	chickpea system	18S rRNA
coix seed	<i>Coix lacryma-jobi</i>	negative	negative	22.2 ± 1.02	negative	18.8 ± 0.08
wild rice	<i>Zizania latifolia</i>	22.9 ± 0.10	negative	negative	negative	17.4 ± 0.07
wild rice	<i>Zizania palustris</i>	23.7 ± 0.69	negative	negative	negative	17.8 ± 0.06
wild rice	<i>Zizania aquatica</i>	25.4 ± 0.60	negative	negative	negative	17.3 ± 0.04
wild rice	<i>Zizania texana</i>	21.9 ± 0.12	negative	negative	negative	17.7 ± 0.07
quinoa (white)	<i>Chenopodium quinoa Willd.</i>	negative	26.2 ± 1.13	negative	negative	13.6 ± 0.13
quinoa (red)	<i>Chenopodium quinoa Willd.</i>	negative	26.1 ± 1.12	negative	negative	15.7 ± 0.07
quinoa (black)	<i>Chenopodium quinoa Willd.</i>	negative	26.4 ± 1.14	negative	negative	14.1 ± 0.11
chickpea	<i>Cicer arietinum L.</i>	negative	negative	negative	24.3 ± 0.79	16.3 ± 1.98
sorghum rice	<i>Sorghum</i>	negative	negative	negative	negative	16.5 ± 0.22
sugarcane	<i>Saccharum officin</i>	negative	negative	negative	negative	18.4 ± 0.14
pseudo sorghum rice	<i>Sorghum halepense (L.) Pers</i>	negative	negative	negative	negative	18.4 ± 0.15
corn	<i>Zea mays L.</i>	negative	negative	negative	negative	15.8 ± 0.11
Chia seed	<i>Salvia Hispanica L.</i>	negative	negative	negative	negative	13.9 ± 0.21
red rice	<i>Lpomoea batatas (L.)</i>	negative	negative	negative	negative	17.7 ± 0.08
black kerneled rice	<i>Semen Trigone</i>	negative	negative	negative	negative	14.8 ± 1.06
rice	<i>Oryza sativa</i>	negative	negative	negative	negative	14.5 ± 0.11
coarse rice	<i>Panicum miliaceum</i>	negative	negative	negative	negative	14.8 ± 0.07
Rye	<i>Secale cereale L.</i>	negative	negative	negative	negative	13.9 ± 2.33
buckwheat	<i>Fagopyrum esculentum Moench.</i>	negative	negative	negative	negative	15.0 ± 0.21
tartary buckwheat	<i>Fagopyrum tataricum (L.) Gaertn.</i>	negative	negative	negative	negative	16.6 ± 0.14
oats	<i>Avena sativa L.</i>	negative	negative	negative	negative	15.1 ± 0.37
barley	<i>Hordeum vulgare L.</i>	negative	negative	negative	negative	16.4 ± 0.10
wheat	<i>Triticum aestivum L.</i>	negative	negative	negative	negative	15.1 ± 0.05
Semen sesami nigrum	<i>Sesamum radiatum</i>	negative	negative	negative	negative	16.6 ± 0.11

black soya bean	<i>Glycine max (L.) merr.</i>	negative	negative	negative	negative	14.1	±	0.14
kidney bean	<i>Phaseolus vulgaris</i>	negative	negative	negative	negative	14.7	±	0.08
soybean	<i>Glycine max (Linn.) Merr.</i>	negative	negative	negative	negative	14.8	±	0.15
Mung bean	<i>Vigna radiata (Linn.) Wilczek.</i>	negative	negative	negative	negative	14.4	±	0.07
hyacinth bean	<i>Lablab purpureus (Linn.) Sweet</i>	negative	negative	negative	negative	12.8	±	0.02
pea	<i>Pisum sativum L.</i>	negative	negative	negative	negative	15.7	±	0.05
red bean	<i>Vigna angularis</i>	negative	negative	negative	negative	12.5	±	0.17
agaric	<i>Auricularia auricula (L.ex Hook.)Uneveerwood</i>	negative	negative	negative	negative	14.8	±	0.19
Xianggu mushroom	<i>Lentinus edodes (Berkeley) Singer</i>	negative	negative	negative	negative	14.6	±	0.15

Table S3. linear equation of qPCR assay of quinoa, coix seed, wild rice anegative chickpea

Event	Linear equation	R ²	Efficiency (E)%
quinoa source component	y = 27.16 - 3.27 Log10 X	0.9923	102.22%
coix seed source component	y = 26.59 - 3.35 Log10 X	0.9984	98.74%
wild rice source component	y = 23.67 - 3.40 Log10 X	0.9965	96.97%
chickpea source component	y = 26.82 - 3.38 Log10 X	0.9989	97.80%

Table S4. Robustness results of quinoa, coix seed, wild rice and chickpea systems

Methods	Ct value of combination								RSD (%)	
	1	2	3	4	5	6	7	8	Mean	Control
Quinoa	35.32 ± 0.11	35.43 ± 0.15	35.45 ± 0.11	35.38 ± 0.15	35.34 ± 0.04	35.36 ± 0.12	35.42 ± 0.15	35.47 ± 0.14	35.40 ± 0.05	35.27 ± 0.05
Coix seed	35.89 ± 0.14	35.92 ± 0.12	35.99 ± 0.11	35.81 ± 0.16	35.94 ± 0.16	35.92 ± 0.14	36.02 ± 0.14	35.96 ± 0.13	35.93 ± 0.06	35.93 ± 0.08
Wild rice	32.73 ± 0.14	32.72 ± 0.22	32.66 ± 0.15	32.74 ± 0.20	33.05 ± 0.18	33.01 ± 0.20	32.97 ± 0.11	32.99 ± 0.11	32.86 ± 0.16	32.82 ± 0.07
Chickpea	35.67 ± 0.10	35.67 ± 0.08	35.80 ± 0.12	35.81 ± 0.11	36.27 ± 0.52	36.00 ± 0.14	36.01 ± 0.19	36.05 ± 0.17	35.91 ± 0.21	35.77 ± 0.05

Table S5. Detection of commercial samples containing quinoa, coix seed, wild rice and chickpea

Sample No.	Sample name	qPCR analysis results (Ct value)			
		Quinoa	Coix seed	Wild rice	Chickpea
1	Coix seed (100%)	negative	23.2 ± 0.08	negative	negative
2	Red bean coix seed Yam powder	negative	32.3 ± 0.12	negative	negative
3	Coix seed red bean Meal replacement cake	negative	negative	negative	negative
4	Red bean coix seed pellet	negative	negative	negative	negative
5	Red bean coix seed Poria cocos cream	negative	36.2 ± 0.11	negative	negative
6	Red bean coix seed steamed bun	negative	36.0 ± 0.04	negative	negative
7	Red bean coix seed tea	negative	33.4 ± 0.14	negative	negative
8	Red bean coix seed Soft Canegativey	negative	35.8 ± 0.21	negative	negative
9	Coix seed noodle	negative	37.5 ± 0.12	negative	negative
10	Red bean coix seed Poria cocos tea	negative	32.7 ± 0.10	negative	negative
11	Chickpea (100%)	negative	negative	negative	24.6 ± 0.09
12	Chickpea noodle (30%)	negative	negative	negative	27.3 ± 0.30
13	Steamed and ready to eat chickpea chips (100%)	negative	negative	negative	24.2 ± 0.08
14	Refined pure raw chickpea leather (100%)	negative	negative	negative	30.6 ± 0.19
15	Canned Turkish chickpea (100%)	negative	negative	negative	37.8 ± 0.07
16	Canned chickpea (100%)	negative	negative	negative	31.3 ± 0.15
17	Fried chickpea (100%)	negative	negative	negative	36.9 ± 0.21
18	Quinoa Bolivia imports quinoa (100%)	26.0 ± 0.17	negative	negative	negative
19	Qinghai 3 colors quinoa (100%)	26.8 ± 0.22	negative	negative	negative
20	Quinoa soda biscuit (3.2%)	negative	negative	negative	negative
21	Quinoa wheat Germ Mixed Cereal (2%)	36.7 ± 0.57	negative	negative	negative
22	Quinoa powder (100%)	26.2 ± 0.08	negative	negative	negative
23	Canada No.1 Ice lake wild rice (100%)	negative	negative	20.7 ± 0.14	negative
24	Wild rice powder (100%)	negative	negative	22.1 ± 0.04	negative