

Analytical Methods

A group-specific, quantitative real-time PCR assay for detection of crab, a crustacean shellfish allergen, in complex food matrices



Anne C. Eischeid*, Sarah R. Stadig

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, 5100 Paint Branch Parkway, College Park, MD 20740, United States

ARTICLE INFO

Keywords:

Crab
Crustacean
qPCR
Real-time PCR
Allergen
Mitochondria
12S rRNA gene
Internal amplification control

ABSTRACT

A real-time PCR assay was developed for detection of crab, a crustacean allergen, in food products. Group-specific primers and probes were developed to detect numerous species of crab. Method validation included tests of detection in complex food matrices, evaluation of commercial food products, and cross-reactivity testing on a wide variety of crustaceans. The method was able to detect several species of crab spiked into complex food matrices at levels ranging from 0.1 to 10^5 parts per million (weight/weight), worked equally well on different platforms, exhibited high specificity for crab over other types of crustaceans, and yielded much higher signals from commercial food products listing crab as an ingredient than from those containing other crustaceans.

1. Introduction

Food allergies affect approximately 15 million Americans, with allergy to crustacean shellfish affecting approximately 6 million, or 2% of the American population (Sicherer, Munoz-Furlong, & Sampson, 2004; Wild & Lehrer, 2005). There is currently no cure or treatment, so affected individuals must avoid the foods to which they are allergic. As a result, consumers with food allergies must rely on proper food labeling to identify the presence of food allergens. To protect allergic consumers, U.S. Congress passed the Food Allergen Labeling and Consumer Protection Act of 2004 (Public Law 108–282, Title II) (FALCPA), requiring the presence of any of the eight major food allergens, including crustacean, be declared on the label.

To enforce FALCPA, accurate and highly sensitive detection assays for allergens in food products are needed, and PCR-based assays represent one of the major methods for detecting food allergens (Sharma, Khuda, Parker, Eischeid, & Pereira, 2017). Conventional end-point PCR has been shown to successfully detect crab at trace levels in several complex food matrices, and performed well in a large multi-laboratory validation (Sakai, Adachi, Akiyama, & Teshima, 2013; Taguchi et al., 2011). Real-time quantitative PCR (qPCR) has proven especially useful for both detection and quantitation of trace levels of allergenic foods with high specificity. Our laboratory has previously reported group-specific qPCR based detection assays for numerous species of shrimp and lobster, as well as one designed for blue crab species (Eischeid, 2016; Eischeid, Kim, & Kasko, 2013).

This report describes the development and evaluation of a group-specific qPCR based assay for detection of trace amounts of crab in food products. The method development and validation reported herein includes previously unpublished qPCR primers and probes which were designed specifically for this work. In this work, the assay was 1) evaluated in complex food matrices using 7 different species of crab from 6 different genera, 2) employed in the analysis of commercial food products, and 3) tested for cross-reactivity using nearly 100 samples and 30 species of crustaceans. Performance of the assay was also compared on 3 different real time PCR systems to confirm the performance was consistent between platforms.

2. Materials and methods

2.1. Primer and probe design

Primers and probes for crab species were designed as previously described for shrimp and lobster (Eischeid, 2016; Eischeid et al., 2013). Full mitochondrial genome sequences were obtained from Genbank for a variety of species including king crabs, mud crabs, blue crabs, other swimming crabs, and a rock crab. Sequences for the 12S rRNA gene were manually extracted from the full mitochondrial genomes. Accession numbers and species used in primer and probe design are as follows: NC_024202 (*Lithodes nintokuae*), NC_020029 (*Paralithodes camtschaticus*), NC_021458 (*Paralithodes brevipes*), NC_012567 (*Scylla tranquebarica*), NC_012572 (*Scylla paramamosain*), NC_012565 (*Scylla*

* Corresponding author.

E-mail address: Anne.Eischeid@fda.hhs.gov (A.C. Eischeid).

Table 1
Evaluation in Complex Food Matrices.

	Linear Range	12S Efficiency	12S R ²	IAC C _T avg.	IAC C _T SD (RSD)
<i>Detection of Crab in a Complex Food Matrix-Macaroni Salad (all PCR on Mx3005P)</i>					
^a Blue Crab	0.1–10 ⁶ ppm	112%	1.00	26.85	0.19 (0.71%)
^a Snow Crab	1–10 ⁵ ppm	101%	1.00	24.64	0.27 (1.1%)
^a Rock Crab	1–10 ⁵ ppm	114%	0.98	34.77	0.32 (0.92%)
^{ab} Red King Crab-Expt 1	0.1–10 ⁶ ppm	102%	0.99	31.75	0.38 (1.2%)
^d Red King Crab-Expt 2	0.1–10 ⁴ ppm	102%	0.96		
^a Blue King Crab	1–10 ⁶ ppm	120%	0.99	30.74	0.31 (1.0%)
^a Red Crab	1–10 ⁶ ppm	130%	0.95	26.76	0.15 (0.56%)
^a Dungeness Crab	0.1–10 ⁶ ppm	96%	0.98	26.78	0.17 (0.63%)
<i>Platform Comparison- Red King Crab in Pork Dumplings</i>					
^b Mx3005P	0.1–10 ⁶ ppm	111%	0.99	28.74	0.16 (0.56%)
^c CFX96-Expt 1	0.1–10 ⁶ ppm	104%	0.98	29.11	0.29 (0.99%)
^d CFX96-Expt 2	0.1–10 ⁴ ppm	98%	0.98		
^c 7500 Fast	0.1–10 ⁶ ppm	100.50%	0.97	29.50	0.32 (1.1%)
<i>Platform Comparison-Red King Crab in Clam Juice</i>					
^b Mx3005P	0.1–10 ⁶ ppm	110%	1.00	28.87	0.19 (0.66%)
CFX96-Expt 1	0.1–10 ⁶ ppm	105%	1.00	28.28	0.36 (1.3%)
^d CFX96-Expt 2	0.1–10 ⁴ ppm	106%	0.99		
7500 Fast	0.1–10 ⁶ ppm	99.40%	0.99	30.63	0.23 (0.75%)

^a used in determining performance across species (Fig.1a)

^b used in determining performance across food matrices (Fig.1b)

^c used in determining performance across platforms (Fig.1c)

^d duplicate independent experiments were carried out to conform to the method validation guidelines set forth by the U.S. FDA (2015). The highest level tested in the duplicate experiments was 10⁴ ppm. Macaroni salad, pork dumplings, and clam juice were purchased from a local market; full ingredient lists are provided in Supplementary Material. Method details are provided in Section 2.2.

serrata), NC_012569 (*Scylla olivacea*), NC_006281 (*Callinectes sapidus*), NC_005037 (*Portunus trituberculatus*), and KJ132446 (*Metacarcinus magister*). All 12S rRNA gene sequences were aligned, and primers and probe were designed from the alignment using Allele ID software (Premier BioSoft, Palo Alto, CA, USA). Primers and probes were designed specifically to bind crab sequences and not to bind 12S rRNA gene sequences from other crustaceans, such as shrimp and lobster. This method of assay design yielded a combination which bound to all species used in design¹: 6 different forward primers (5′-3′ sequences: TCTTTCCAGGCTTACTTTCCAG, GGCACATCTACTTTGTTACG, TAAACCTACTATGTTACGACTTATCTC, CACCTACTTTGTTACGACTTATTC, CCTACTATGTTACGACTTATCTC, GCTTATAAATAAGTTAAAGTGGGT-TAC), 8 different reverse primers (5′-3′ sequences: CTTAGTTAGATAT-AAGCTCTAAATCATG, GCTAATTAGATATAAGCTCTAAAGCATG, AAT-TACTTGATATAGGCTCTAAAATATGTAC, AATTTAATATGTTTAGTTA-GATATAAGC, AAATTTAATATGTTTAAATTAGATATAAGC, ATTAATT TTAATTACTTGATATAGGC, AATAGTTTAGTATGGTATTTAGATATAG-GC, TGTACATGATTTAGAACTTATATCTC), and a single probe (5′-FAM-ACATATCGC-ZEN-CCGTCGCTTTC-3′-IowaBlack FQ). Species-specific information regarding individual primer and probe sequences is provided as Supplementary Material. All PCR products were approximately 100 bp in size, with minor variations depending on species. An exogenous internal control developed by Deer, Lampel, and Gonzalez-Escalona (2010) was used as previously described (Eischeid, 2016; Eischeid et al., 2013). Primers, probes, and ultramer DNA template for the internal control were purchased from Integrated DNA Technologies (Coralville, IA).

2.2. Evaluation in complex food matrices

Sample preparation, DNA extraction, and PCR were carried out as previously described with minor modifications (Eischeid, 2016; Eischeid et al., 2013). For method evaluation in foods, crab meat was spiked at levels of 0.1, 1, 10, 100, 1000, 10⁴, and 10⁵ ppm (w/w) into

three commercial food products obtained from a local market: pork dumplings, macaroni salad, and clam juice (Table 1; full ingredient lists are provided in Supplementary Material). In order to ensure accurate spiking at the very low levels used in this work, homogenates containing known amounts of crab meat were prepared and these homogenates were added to foods. All experiments included a matrix blank consisting of food matrix with no crab, a homogenization blank consisting of only buffer with no food matrix and no crab, and an extraction blank consisting only of buffer and introduced at the DNA extraction step. In most experiments, a sample consisting entirely of crab DNA (10⁶ ppm, w/w) was included as a positive control not subject to the effects of the food matrix. Samples were homogenized using 5 g sample in 20 ml of 1–2% SDS buffer at 10,000–12,000 rpm for 30–40 s using an IKA Tube Mill (IKA, Wilmington, NC). As some of the milling tubes leaked, homogenization tests were carried out to confirm that leakage did not cause contamination of subsequent samples (data not shown). DNA was extracted from 200 µl of homogenate using a combined rapid salt extraction (Aljanabi & Martinez, 1997) and the DNeasy kit (catalog No. 69506, Qiagen, Valencia, CA) as previously described (Eischeid, 2016; Eischeid et al., 2013). DNA was eluted in 60 µl Buffer AE and concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE). For macaroni salad and pork dumplings, template DNA samples were diluted to 10 ng/µl in water and 5 µl (50 ng total DNA) was used per reaction in PCR. For clam juice, template DNA was used undiluted at 5 µl per reaction because DNA concentrations were below 10 ng/µl in this food matrix. Real time PCR was carried out as described in Section 2.5.

2.3. Cross-reactivity testing

Cross-reactivity testing was carried out using nearly 100 samples from 30 species of crustaceans (Table 2). For these tests, DNA was extracted directly from crustacean tissue using the DNeasy kit (Qiagen, Valencia, CA) as per manufacturer's instructions. All DNA samples were diluted to 100 pg/µl in water and 5 µl (500 pg total DNA) was used per reaction; this is equivalent to the amount of crab DNA present in a 10⁴ ppm-spiked sample. PCR was carried out as described in Section 2.5 on the Mx3005P platform, and data were analyzed for C_T value and

¹ Primer sequences in italics represent primers which were inadvertently included in PCR. These two primers target a different part of the 12S rRNA gene from that which the probe targets and likely did not affect assay performance.

Table 2
Cross-Reactivity Testing.^a

Common/MarketName	Scientific Name	C _T value	dRn Last ^b
<i>Crabs</i>			
Dungeness Crab	<i>Metacarcinus magister</i>	31.14	0.90
Dungeness Crab	<i>Metacarcinus magister</i>	28.10	1.70
Dungeness Crab	<i>Metacarcinus magister</i>		NA
Blue Crab	<i>Callinectes sapidus</i>	28.16	4.97
Blue Crab	<i>Callinectes sapidus</i>	25.65	5.58
Blue Crab	<i>Callinectes sapidus</i>	27.43	5.34
Blue Crab	<i>Callinectes sapidus</i>	24.36	5.90
Blue Crab	<i>Callinectes sapidus</i>	21.31	6.20
Blue Crab	<i>Callinectes sapidus</i>	21.00	6.24
Jonah Crab	<i>Cancer borealis</i>	42.34	0.09
Jonah Crab	<i>Cancer borealis</i>		
Jonah Crab	<i>Cancer borealis</i>		
Snow Crab	<i>Chionoecetes opilio</i>	35.82	1.75
Snow Crab	<i>Chionoecetes opilio</i>	no C _T	NA
Snow Crab	<i>Chionoecetes opilio</i>	31.67	1.75
Snow Crab	<i>Chionoecetesopilio</i>	26.54	5.82
Snow Crab	<i>Chionoecetes opilio</i>	27.17	5.28
Southern King Crab	<i>Lithodes santolla</i>	26.88	4.52
Red King Crab	<i>Paralithodes camtschaticus</i>	28.46	4.05
Red King Crab	<i>Paralithodes camtschaticus</i>	30.07	3.64
Red King Crab	<i>Paralithodes camtschaticus</i>	27.75	4.48
Red King Crab	<i>Paralithodes camtschaticus</i>	29.58	3.71
Red King Crab	<i>Paralithodes camtschaticus</i>	23.59	6.40
Blue King Crab	<i>Paralithodes platypus</i>	29.51	4.29
<i>Crayfish</i>			
Red Swamp Crayfish	<i>Procambarus clarkii</i>	no C _T	NA
Red Swamp Crayfish	<i>Procambarus clarkii</i>	no C _T	NA
<i>Lobsters</i>			
(lobster tail)	NA	40.64	0.14
(lobster tail)	NA	41.24	0.12
Squat Lobster	<i>Cervimunida johnei</i>	no C _T	NA
American Lobster	<i>Homarus americanus</i>	34.08	1.19
American Lobster	<i>Homarus americanus</i>	no C _T	NA
American Lobster	<i>Homarus americanus</i>	34.24	1.57
Southern Rock Lobster	<i>Jasus edwardsii</i>	no C _T	NA
Southern Rock Lobster	<i>Jasus edwardsii</i>	no C _T	NA
St. Paul Spiny Lobster	<i>Jasus paulensis</i>	no C _T	NA
St. Paul Spiny Lobster	<i>Jasus paulensis</i>	no C _T	NA
St. Paul Spiny Lobster	<i>Jasus paulensis</i>	no C _T	NA
St. Paul Spiny Lobster	<i>Jasus paulensis</i>	no C _T	NA
Florida Lobsterette	<i>Nephropsis aculeata</i>	30.94	4.5
Western Red Lobster	<i>Panulirus cygnus</i>	no C _T	NA
Western Red Lobster	<i>Panulirus cygnus</i>	no C _T	NA
(lobster)	<i>Panulirus delagoae</i>	42.91	0.68
(lobster)	<i>Panulirus delagoae</i>	no C _T	NA
(lobster)	<i>Panulirus delagoae</i>	no C _T	NA
<i>Lobsters (cont.)</i>			
(lobster)	<i>Panulirus delagoae</i>	no C _T	NA
Smoothtail Spiny Lobster	<i>Panulirus laeviscauda</i>	no C _T	NA
Smoothtail Spiny Lobster	<i>Panulirus laeviscauda</i>	no C _T	NA
Smoothtail Spiny Lobster	<i>Panulirus laeviscauda</i>	no C _T	NA
Smoothtail Spiny Lobster	<i>Panulirus laeviscauda</i>	no C _T	NA
Ornate Spiny Lobster	<i>Panulirus ornatus</i>	no C _T	NA
Ornate Spiny Lobster	<i>Panulirus ornatus</i>	no C _T	NA
Colorado Langostino	<i>Pleuroncodes monodon</i>	no C _T	NA
Ridges Slipper Lobster	<i>Scyllarides nodifer</i>	32.40	1.06
slipper lobster	<i>Thenus sp.</i>	33.41	0.83
<i>Shrimp</i>			
Brown Shrimp	<i>Farfantepenaeus aztecus</i>	no C _T	NA
Brown Shrimp	<i>Farfantepenaeus aztecus</i>	no C _T	NA
Brown Shrimp	<i>Farfantepenaeus aztecus</i>	no C _T	NA
Brown Shrimp	<i>Farfantepenaeus aztecus</i>	37.47	0.35
Brown Shrimp	<i>Farfantepenaeus aztecus</i>	no C _T	NA
Pink Shrimp	<i>Farfantepenaeus duorarum</i>	34.30	1.93
shrimp	<i>Farfantepenaeus sp.</i>	no C _T	NA
White Shrimp	<i>Litopenaeus setiferous</i>	no C _T	NA
White Shrimp	<i>Litopenaeus setiferous</i>	no C _T	NA
White Shrimp	<i>Litopenaeus setiferous</i>	no C _T	NA

(continued on next page)

Table 2 (continued)

Common/Market Name	Scientific Name	C _T value	dRn Last
White Shrimp	<i>Litopenaeus setiferous</i>	no C _T	NA
White Shrimp	<i>Litopenaeus setiferous</i>	33.30	1.89
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	no C _T	NA
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	no C _T	NA
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	36.92	0.41
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	no C _T	NA
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	38.64	0.28
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	38.91	0.28
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	35.48	0.85
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	no C _T	NA
Whiteleg Shrimp	<i>Litopenaeus vannamei</i>	no C _T	NA
Giant Freshwater Prawn	<i>Macrobrachium rosenbergii</i>	no C _T	NA
Giant Freshwater Prawn	<i>Macrobrachium rosenbergii</i>	no C _T	NA
Royal Red Shrimp	<i>Pleoticus robustus</i>	no C _T	NA
Brown Rock Shrimp	<i>Sicyonia brevirostris</i>	38.65	0.34
(shrimp)	NA		NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA
(shrimp)	NA	no C _T	NA

Method details are provided in Section 2.3.

^a All samples with scientific names listed in Table 2 were identified using DNA barcoding (Sec. 2.6).

^b dRn Last is the maximum, final fluorescence level obtained at the end of cycling.

maximum final fluorescence (dRn Last).

2.4. Analysis of commercial food products

For market samples, a standard curve was generated by spiking red king crab meat into pork dumplings at 0.1, 1, 10, 100, 1000, 10⁴, and 10⁵ ppm(w/w) as described in Section 2.2. The food samples were homogenized directly using 5 g of food sample and 20 ml 1% SDS, with no addition of crab meat. Homogenization and DNA extraction for both standards and food samples were carried out as described in Section 2.2. All samples with DNA concentrations above 10 ng/μL were diluted to 10 ng/μL, those with lower concentrations were used undiluted. PCR was carried out using the Mx3005P as described in Section 2.5; food samples were treated as unknowns and their concentrations were determined by interpolation from the standard curve in pork dumplings using the software provided by the manufacturer (Table 3).

2.5. Real-Time PCR

PCR was carried out as previously described with minor modifications (Eischeid, 2016; Eischeid et al., 2013). Each PCR mixture contained the following in a 12.5 μL reaction volume: 1 × PCR buffer, 5 mM Mg, 30 nM ROX reference dye, 1.2 mM dNTPs, 0.625 units Platinum Taq polymerase (Invitrogen/Life Technologies, Grand Island, NY, USA), 0.2 μM forward IAC primer, 0.2 μM reverse IAC primer, 0.2 μM IAC probe, 5 × 10⁵ copies IAC template, 1 μM each crab primer, and 0.2 μM crab probe. All primers were included in the PCR reaction mix. Thermal cycling consisted of an initial denaturation at 95 °C for 3 min followed by 45 cycles of denaturation (95 °C, 15 s), annealing (65 °C, 30 s), and extension (65 °C, 2 min). Fluorescent signals were read at the end of the annealing step during each cycle. PCR was carried out on three different platforms, including the Mx3005P (Agilent Technologies, Santa Clara, CA), the 7500 Fast (Applied Biosystems/Life Technologies, Carlsbad, CA) and the CFX 96 (BioRad, Hercules, CA). Data obtained using each platform were analyzed with accompanying software provided by the manufacturers. Data were used to generate standard curves and analyzed as previously described (Eischeid, 2016; Eischeid et al., 2013). For the crab 12S target, statistical R² values were calculated for the best-fit line, and reaction efficiency was determined

from its slope using the equation $E = 10^{(-1/m)} - 1$, where m = slope of the linear standard curve (Adams, 2006). Since the internal control is expected to yield consistent C_T values in all experiments, average and standard deviation were calculated for the IAC at all concentration levels in a given experiment.

2.6. Species identification

Identification of crustacean samples was carried out via DNA barcoding of the COI gene. DNA barcoding is a widely accepted method of species identification based on PCR amplification and sequencing of a standardized, 655 basepair segment of the mitochondrial COI gene. The method used to identify samples for this work has been specifically optimized and validated for use in identification of crustacean shellfish (Eischeid, Stadig, Handy, Fry, & Deeds, 2016). This DNA barcoding procedure was used to identify all crab species used for spiking into complex food matrices as well as shrimp, crab and lobster species used in cross-reactivity testing.

3. Results and discussion

3.1. Evaluation in complex food matrices

Results demonstrated that the assay was able to detect 7 different species of crab from 6 different genera, all at trace levels in complex food matrices (macaroni salad, pork dumplings, clam juice). Data were analyzed by determining C_T values from amplification plots and graphing C_T as a function of crab ppm (w/w) level to generate standard curves (Codex Commission, Validation of Methods for the Detection, & Quantification of Specific DNA Sequences, 2010; ENGL, 2015). Reaction efficiencies were calculated from the slopes of the standard curves as per Section 2.5. Experiments were carried out to determine performance of the method across species, across food matrices, and across platforms. Performance across species was determined using blue crab (*Callinectes sapidus*), snow crab (*Chionoecetes opilio*), rock crab (*Cancer borealis*), red king crab (*Paralithodes camtschaticus*), blue king crab (*Paralithodes platypus*), red crab (likely *Portunus* or *Chaceon* sp.), and Dungeness crab (*Metacarcinus magister*). Each of these species was detected in macaroni salad with linearity over 6–8

Table 3
Analysis of Commercial Food Products.

Description	Allergen-Related Labeling	ppm
<i>Crab as an Ingredient</i>		
crab soup ^a	crab as an ingredient, dried crab as an ingredient; may contain traces of wheat	2.65×10^1
canned crab meat	crab meat as an ingredient	8.52×10^2
canned crab meat	crab meat as an ingredient	1.16×10^3
crab & corn chowder	crab, soybean, pollock, dried crab, cod extract, clam extract, and shrimp extract as ingredients.	9.58×10^2
crab & corn chowder	crab meat, concentrated crab flavor, rock crab, crab roe, cod liver oil, lobster extract, crab flavor, natural crab extract as ingredients; contains wheat, soy, milk, shellfish, and cod	2.90×10^1
crab soup ^a	crab as an ingredient, dried crab as an ingredient; may contain traces of wheat	4.20
cream of crab soup	fish stock (haddock), light cream (milk and cream), crab meat, sulfites, crab extract, clam broth (wild harvested clams and clam broth), worcestershire sauce (anchovies) as ingredients; contains milk, crustacean shellfish (crab), fish (haddock, anchovy)	1.38×10^4
crab and spinach dip	crab meat, sour cream (light cream and nonfat milk), cream cheese (cultured milk and cream), parmesan cheese (part-skim cow's milk), gouda cheese (cultured pasteurized milk), worcestershire sauce (anchovies) as ingredients; contains milk, crustacean shellfish (crab), fish (anchovies)	1.60×10^5
crab soup	crab meat, crab extract as ingredients; contains crustacean shellfish (crab)	1.40×10^3
imitation crab	alaska pollock, king crab meat, natural and artificial flavor (extracts of blue crab, snow crab, lobster, and alaska pollock), refined fish oil (anchovy and sardine), egg whites, soy lecithin, carmine as ingredients; contains fish (alaska pollock, anchovy, sardine), crustacean shellfish (king crab, blue crab, snow crab, and lobster), egg, and soy	1.05×10^2
crawfish cakes	Louisiana crawfish tail meat, imitation crabmeat (pollack, cod, and/or pacific whiting), wheat starch, snow crab meat, soy, egg whites, carmine, cream cheese (past. milk), soybean oil, nonfat dry milk, soy protein, anchovies as ingredients; contains louisiana crawfish tail meat, egg, fish, milk, shellfish, soy, wheat	0.97
crabcakes	crabmeat, imitation crabmeat (pollack, cod, whiting, wheat starch, snow crab meat, flavors/soy, egg whites, carmine, soy lecithin), pollack, wheat flour, cream cheese (past milk and cream), soybean oil, nonfat dry milk, hydrolyzed soy proteins, anchovies; contains crustacean shellfish (crab), fish (pollock, cod, whiting, anchovies), wheat, milk, eggs, and soy	7.25×10^3
crabcakes cajun style	crabmeat, imitation crabmeat (pollack, cod, whiting, wheat starch, snow crab meat, flavors/soy, egg whites, carmine, soy lecithin), pollack, wheat flour, cream cheese (past milk and cream), whey, soybean oil, nonfat dry milk, hydrolyzed soy and corn proteins, anchovies; contains: allergens crustacean shellfish (crab), fish (pollock, cod, whiting, anchovies), eggs, wheat, milk, and soybean	4.56×10^2
crabcakes	crabmeat, mayo (soybean oil, egg) bread crumbs (wheat flour), fish protein (pollock, cod, whiting, snow crab), worcestershire sauce (anchovies), flavor/soy; contains crab, fish, wheat, eggs, soy	5.94×10^4
crab cakes	crab meat, soybean oil, egg, worcestershire sauce (anchovies), wheat flour; contains crustacean shellfish (crab), egg, fish (anchovies), wheat	3.74×10^5
crab crostini	100% real crab meat. Wheat flour, barley, past milk, milk fat, whey, crab, past cream, soybean oil, whole eggs; contains wheat, eggs, milk, soy, crustacean shellfish	3.51×10^4
lobster bites	minced lobster, imitation lobster (fish protein, egg whites, soybean oil, crab flavor, natural crab extract, carmine) Monterey jack cheese (past milk), imitation monterey jack cheese (part hydrog soybean oil casein), low moisture part skim mozzarella cheese (part skim milk), wheat flour, creamer (sodium caseinate/milk derivative, soy lecithin), whole eggs, cooked lobster meat, butter (past cream), clam broth; contains: milk, egg, soy, wheat, fish (pollack, whiting, cod, bream), crustacean (crab and lobster)	1.35×10^2
<i>Lobster as an Ingredient</i>		
lobster bisque	100% North Atlantic Lobster; lobster, lobster base (lobster meat, etc), lobster tomalley, gelatin (perch, tilapia), shrimp powder, lobster extract as ingredients; contains lobster, shrimp, perch, tilapia, milk, wheat, sulfites	0.00
Lobster bisque	seafood stock (clam broth from concentrate, lobster concentrate/lobster, fish concentrate/pollack and codfish, light cream, lobster, wheat flour as ingredients; contain milk, lobster, wheat, and fish (pollack and cod)	7.49
ricotta and lobster ravioli	durham wheat flour, ricotta cheese (whey, cream), egg, lobster, bread crumbs (wheat), lobster powder, soybean oil, whey protein concentrate, clam extract concentrate as ingredients; contains eggs, milk, shellfish (lobster), wheat	7.20
lobster cakes with dipping sauce	lobster meat, lobster surimi (fish protein, pollack and or whiting, wheat, soybean oil, natural and artificial lobster flavor), mayo (soybean oil and egg yolk and wheat flour), butter (cream), bread crumbs (wheat flour), soy protein concentrate, hydrolyzed soy protein; contains lobster, pollack and/or whiting, egg, milk, soy, and wheat. dipping sauce contains soy and milk	19.30
lobster mac and cheese	pasta (wheat, semolina), egg whites, alfredo sauce (cream powder, nonfat dry milk, past milk and cream), lobster; contains milk, egg, soy, wheat, and crustacean (lobster)	11.15
lobster stuffed mushrooms	100% North Atlantic Lobster. Lobster, skim mozz cheese (past milk), wheat flour, part hydrog soybean oil, whey, soy flour, wheat gluten, nonfat dry milk, soy lecithin, past sheeps milk, hydrol soy protein; contains wheat, milk, soy, crustacean shellfish, egg components, barley	8.62
ricotta and lobster ravioli in seafood bisque	wheat flour, whey, cream, egg, lobster, lobster powder, cooked shrimp, butter (cream), soybean oil, shrimp shell powder, shrimp powder, cod powder, fish powder (cod, blue whiting, haddock, pollock, redfish, halibut); contains egg, fish (blue whiting, cod, haddock, halibu, pollock, redfish), milk, shellfish (lobster and shrimp), wheat	2.47
<i>Shrimp as an Ingredient</i>		
canned med. shrimp	shrimp as an ingredient	0.13
canned tiny shrimp	shrimp as in ingredient	0.20
cream of shrimp soup	shrimp, soybean, wheat as ingredients	0.00
bowl noodle soup, spicy kimchi flavor	anchovy, shrimp as ingredient in dried kimchi flakes, sand lance concentrate, seaweed extract, shrimp, soybeans as ingredients; contains wheat, anchovy, shrimp, soy, and sand lance (fish); manuf in a facility that also processes milk.	0.00
cup of noodles with shrimp	dried shrimp, shrimp extract powder, shrimp powder as ingredients; contains wheat, soybean, egg, milk, and shrimp; manuf in a facility that also processes peanut, tree nuts, crustacean shellfish, and fish products	0.66
shrimp and corn chowder	light cream, shrimp, wheat flour, soybean oil, soy lecithin, shrimp stock (shrimp) as ingredients; contains milk, shrimp, wheat, and soy.	0.72
shrimp scampi	cooked pasta (wheat flour), creamy garlic sauce (cream, milk solids, whey powder, soy sauce powder), shrimp as ingredients; contains shrimp, wheat, milk, and soy	0.00
shrimp and vegetable stir fry dinner	shrimp, soy sauce, wheat as ingredients; contains shellfish (shrimp), soy wheat	0.04
shrimp and vegetable dinner	raw shrimp, soy sauce (wheat, soybeans), soybean oil, soy lecithin; contains shellfish(shrimp), soy, and wheat	0.00
shrimp primavera	cooked pasta(durham wheat flour),mild marinara sauce (milk solids, soy sauce powder, whey powder, shrimp as ingredients; contains shrimp, wheat, milk, soy)	0.00
buffalo popcorn shrimp	shrimp, soybean oil, bleached wheat flour, wheat gluten, egg whites; contains shrimp, wheat and egg	0.00

(continued on next page)

Table 3 (continued)

Description	Allergen-Related Labeling	ppm
coconut popcorn shrimp	shrimp, soybean oil, bleached wheat flour, coconut, wheat gluten, egg whites, partially hydrog, soybean oil, nonfat milk, whey; contains shrimp, wheat, tree nuts (coconut), milk eggs, and soy	0.00
shrimp alfredo	skim milk, shrimp, cream, parmesan cheese (cult milk, past milk), romano cheese (cult cows milk), wheat flour, whey, soybean oil, wheat gluten; contains shrimp, wheat and milk ingredients; partially prod with genetic engineering	0.01
pasta in garlic sauce with shrimp and vegetables	wheat, semolina, soybean oil, whey solids, milkfat, raw shrimp; contains milk, wheat, soy, and crustacean shellfish (shrimp)	0.39
beer battered shrimp	shrimp, beer, soybean oil, wheat flour, whey; contains shrimp, wheat, milk	0.01
<i>Precautionary Labeling</i>		
smoky bacon crab dip	imitation crab meat (pollack and/or whiting), wheat starch, natural and artificial crab flavors, soybean oil, soy lecithin, cheese, sour cream, whey, soy flour as ingredients; contains pollack, whiting, snow crab, eggs, wheat, soy, milk.	0.00
seafood salad	imitation crab meat (pollack and/or whiting), wheat starch, natural and artificial crab flavors, soybean oil, soy lecithin, egg yolk as ingredients; contains alaskan pollack, pacific whiting, snow crab, eggs, wheat, soy, milk	0.00
noodles, chicken flavor	contains wheat, soybean; manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, and fish	0.04
noodles, oriental flavor	contains wheat, soybean; manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, and fish	0.04
noodles & soup, original	manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, and fish products	0.00
noodles & soup, chicken flavor	manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, fish, soybeans, and sesame products	0.00
dry noodles, shrimp flavor	shrimp extract powder and shrimp extract powder as ingredients; contains: wheat, soybean, and shrimp; manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, and fish products	1.09
dry noodles, chili flavor	contains wheat and soybean; manuf in a facility that also processes egg, milk, peanut, tree nuts, crustacean shellfish, fish	0.01
dry noodles, spicy seafood flavor	anchovy extract, anchovy, mussel, cuttlefish extract, sand lance extract, tuna extract, bonito extract as ingredients; contains wheat, soy, anchovy, tuna, and sand lance(fish); manuf. in a facility that also processes crustacean shellfish and milk	0.00
dry noodles, spicy seafood flavor	anchovy extract, anchovy, mussel, cuttlefish extract, sand lance extract, tuna extract, bonito extract as ingredients; contains wheat, soy, anchovy, tuna, and sand lance(fish); manuf. in a facility that also processes crustacean shellfish and milk	0.00
bowl of noodle soup, spicy shrimp flavor	cuttlefish, freeze-dried shrimp, mussel, oyster, sand lance concentrate, sea squirt, seaweed extract, shrimp, tuna as ingredients; contains wheat, soy, sand lance (fish), shrimp, tuna; manuf in a facility that also processes milk	0.59
bowl of noodle soup, gourmet spicy flavor	anchovy extract, soybean sprout extract as ingredients; contains wheat, anchovy, and soy; manuf. in a facility that also processes crustacean shellfish and milk	0.00
bowl of noodles soup, hot and spicy flavor	dried fish cake (pollack and bream) as ingredients; contains wheat, soy, pollack, and bream (fish); manuf. in a facility that also processes crustacean shellfish and milk	1.86
bowl of noodles, spicy pot-au-feu flavor	anchovy extract, seaweed extract as ingredients; contains wheat, anchovy, and soy; manuf. in a facility that also processes milk and crustacean shellfish	1.81
seafood stock	anchovy, haddock, cod, pollack, salmon, sole, flounder as ingredients; panel on side says that ingredients must not contain milk, eggs, peanuts, glutens, or tree nuts. Each production run is tested for the absence of these allergens to 5 ppm.	0.00
New England Clam Chowder	clam stock, clams, flavoring (cod), clam extract, whey, partially hydrog soybean oil, soy lecithin, soybeans, whey protein concentrate, nonfat dry milk, cream, what flour, soy protein concentrate, soy sauce as ingredients	0.27
New England Clam Chowder	light cream, fish stock (pollack), clam meat, wheat flour, clam broth powder (clam broth), worcestershire sauce (anchovies) as ingredients; contains milk, fish (pollack, anchovy) and wheat	0.10
cream of shrimp soup	shrimp, soybean, wheat as ingredients	0.20
shrimp fried rice	cooked shrimp, soybean oil, dehydrated soy sauce, oyster flavored sauce(oyster extractives) as ingredients; contains shellfish (oyster and shrimp), wheat, soy	0.00
shrimp spring rolls	flour, soybean oil, fish sauce(anchovies), oyster sauce (soy sauce/soybeans, oyster extractives, oyster flavor); contains shrimp, anchovies, wheat, soy	0.35
oyster flavored sauce	oyster extract (water, oyster, salt), fish paste (crustacean shellfish); this product is manuf in a facility that uses tree nuts, peanuts, celery, mustard, and sesame seeds	0.02

^a These two samples of crab soup were both canned and tomato-based products; method details for data in this table are provided in Section 2.4.

orders of magnitude, statistical R^2 values very close to 1.00 in most cases, and lower limits of detection ranging from 0.1 to 10 ppm, w/w (Table 1; Fig. 1a). Reaction efficiencies ranged from 99 to 130%; some of these are outside the preferred, ideal range of 90–110% and also outside the range we have obtained with our previous assays for shrimp and lobster species (Codex Alimentarius Commission, 2010; Eischeid, 2016; Eischeid et al., 2013; ENGL, 2015). This may be because we have tested a wider range of species in the current paper, or because the genetic variability in crabs has made it more challenging to obtain a group-specific assay which performs with ideal reaction efficiencies in a variety of species. Nonetheless, these data show that the method detects trace levels of at least 7 different crab species from 6 different genera across 6–8 orders of magnitude in a single assay. Average C_T values for experiments across species yield a standard curve with reaction efficiency of 113%, R^2 coefficient of 0.99, and relative standard deviations (RSDs) of 10–17% (Fig. 1a).

Performance of the method across food matrices was determined using data from the Mx3005P platform with red king crab spiked into macaroni salad, pork dumplings, and clam juice (Table 1). Average C_T values for these data yield a standard curve with reaction efficiency of 108%, an R^2 of 0.99, and RSD values ranging from 2 to 10% (Fig. 1b). Performance across platforms was determined using data for red king crab spiked into pork dumplings and clam juice, and PCR was carried out on CFX96, Mx3005P, and 7500 Fast platforms (Table 1). As an example of performance across platforms, data for pork dumplings were averaged to yield a combined reaction efficiency of 104%, R^2 of 0.98, and RSD values ranging from 2 to 10% (Fig. 1c). Reaction efficiencies and R^2 values are within the ideal ranges across both food matrix and platform (Codex Alimentarius Commission, 2010; ENGL, 2015).

The internal control yielded some inconsistencies in C_T value from one independent experiment to the next (Table 1). Separate tests carried out using only the internal control indicate the IAC amplifies with

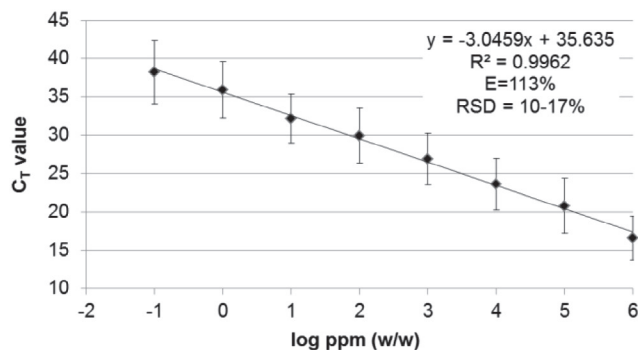
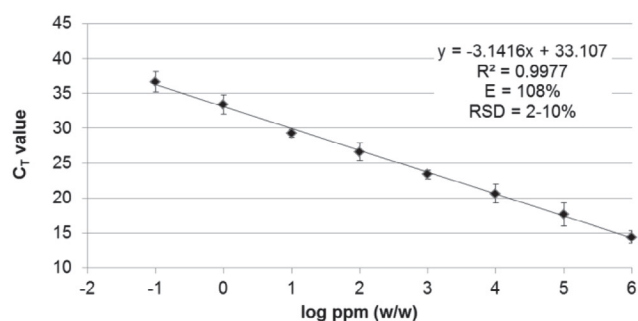
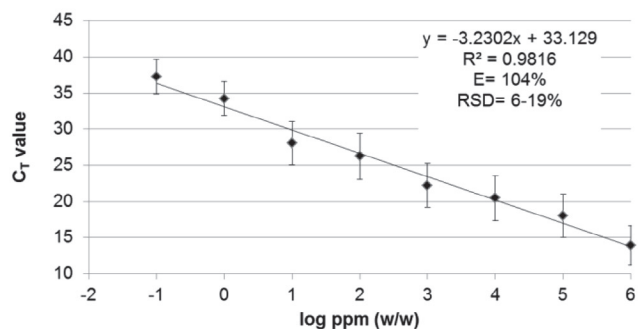
a: Assay performance across species**b: Assay performance across food matrices****c: Assay performance across platforms**

Fig. 1. Average C_T values are plotted as a function of ppm (w/w) spiking level to examine method performance across species (1a), across food matrices (1b), and across platforms (1c) as discussed in Section 3.1. Error bars show 1 standard deviation (SD) above the mean and 1 SD below the mean. Slopes, y-intercepts, reaction efficiencies, and R^2 coefficients were determined using the best fit line through the average C_T values. Performance data for individual experiments are provided in Table 1.

high reaction efficiencies under the conditions used in this study even when the C_T values vary from one independent test to the next, and that the presence of crab primers and probes does not interfere with amplification of the IAC (data not shown). Furthermore, the IAC amplified very consistently within any given experiment, with a maximum relative standard deviation (RSD) of 1.2% (Table 1).

3.2. Cross-reactivity testing

Cross-reactivity testing was carried out using DNA extracted from nearly 100 samples, including approximately 10 different species of each type of crustacean (shrimp, crab, and lobster) in order to ensure that species from the intended target group were detected while closely related nontarget species were not (Codex Alimentarius Commission, 2010; ENGL, 2015). Results of the cross reactivity testing show that, in

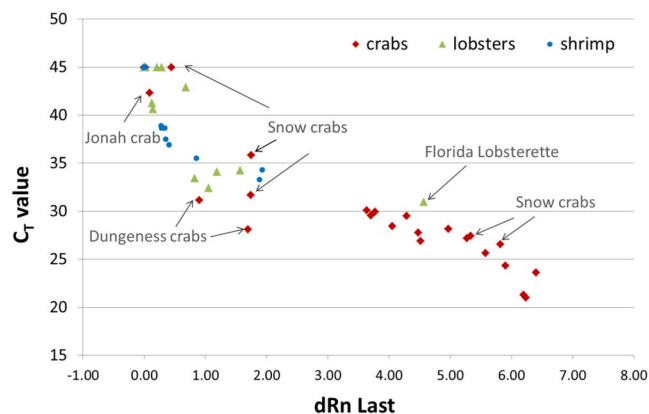


Fig. 2. Results of cross-reactivity testing. Plot of C_T value versus dRn last (maximum fluorescence at the end of cycling) for samples used in cross-reactivity testing. In this plot, stronger positive signals are associated with lower C_T values and higher dRn levels. Including dRn, the level of fluorescence at the end of the last cycle of PCR gives a further indicator of signal strength and helps distinguish strong positive signals more readily than C_T value alone.

general, the assay yielded strong signals from various crab species and little to no signal from shrimp, lobster, and crayfish species (Table 2 and Fig. 2). The only substantial cross-reactivity we observed was with Florida Lobsterette (*Nephropsis aculeata*), which gave strong signals and clearly grouped with crabs in a plot of C_T values and final fluorescence level (Fig. 2). Among crabs, Dungeness crab and Jonah crab did not give strong signals and snow crab gave inconsistent signals in cross-reactivity tests (Fig. 2). All three of these species, however, were detected when spiked into a complex food matrix (Table 1). Inconsistencies in detection may reflect a stabilizing effect of background DNA from the food matrix or variation in the samples themselves. In these tests, we used samples which were purchased commercially and stored in the laboratory for different periods of time under different conditions. We did this in order to obtain a wide variety of species and numerous individuals of a given species, when available, but sample age and storage conditions of the samples likely affected the PCR results. The assay would also likely detect *Scylla* and *Portunus* species, as sequences from these genera were included in assay design (Section 2.1) but samples from them were not included in cross-reactivity testing. The internal control (IAC) amplified consistently at C_T values of approximately 23–26 in the cross-reactivity tests.

3.3. Analysis of commercial food products

We tested the assay using 60 samples purchased from a local market which either had some type of crustacean listed as an ingredient or carried precautionary labeling with respect to crustacean allergens. All samples which contained crab as a labeled ingredient gave positive results (Table 3). The wide variation in concentration from products which contained crab as an ingredient can be explained by several factors, including variation in the amount of crab contained in the product or in the subsample taken for analysis, processing of the product, and quality of the product. The canned crab meats and products which contained tomato-based (acidic) ingredients gave lower signals than might be expected; however, it is known that both acidic conditions and canning can adversely affect DNA integrity and in turn real time PCR assays like the one used here (Bauer, Weller, Hammes, & Hertel, 2003; Bergerova, Godalova, & Siekel, 2011; Eischeid, 2016; Hird et al., 2006). Of the samples which carried precautionary labeling or contained shrimp as an ingredient, all had less than 2 ppm crab and only three had greater than 1 ppm (Table 3). All but one of the foods containing lobster as an ingredient gave positive signals, though they were substantially lower than those obtained from products containing crab. Our cross-reactivity results, reported above,

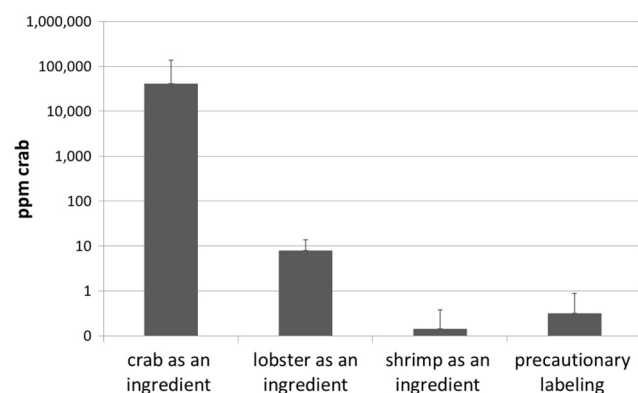


Fig. 3. Results obtained from market samples. Levels of crab detected in market samples. Plot shows average ppm levels for each group of products tested; error bars show 1 SD above and 1 SD below the mean.

indicate that Florida Lobsterette may give a strong signal with this assay, and these lobster products might have contained that species or a similar one. We note that one sample specified North Atlantic lobster (which is not a lobsterette), and that no signal was detected in this sample (Table 3). Average concentrations from all samples in each group indicate that the products containing crab gave signals that were 4–5 orders of magnitude greater than those which did not contain crab (Fig. 3). In general, then, market samples which contained crab as a labeled ingredient gave strongly positive signals compared to samples which contained shrimp or lobster as labeled ingredients. These data indicate that the assay works effectively in market samples which have been subjected to the preparation and processing conditions used by the food industry, and that crab can be differentiated from other crustaceans in these samples. The internal control (IAC) amplified consistently at C_T values of approximately 23–25 for all market samples.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was funded by the United States Food and Drug Administration.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the

online version, at <http://dx.doi.org/10.1016/j.foodchem.2017.10.045>.

References

- Adams, P. S. (2006). Data Analysis and Reporting. In M. T. Dorak (Ed.). *Real-time PCR* (pp. 39–62). New York, NY: Taylor and Francis.
- Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25(22), 4692–4693.
- Bauer, T., Weller, P., Hammes, W. P., & Hertel, C. (2003). The effect of processing parameters on DNA degradation in food. *European Food Research and Technology*, 217, 338–343.
- Bergerova, E., Godalova, Z., & Siekel, P. (2011). Combined effects of temperature, pressure, and low pH on the amplification of DNA of plant derived foods. *Czech Journal of Food Science*, 29(4), 337–345.
- Codex Commission, “Guidelines on Performance Criteria and Validation of Methods for the Detection, Identification, and Quantification of Specific DNA Sequences and Specific Proteins in Foods Standard CAC/GL 74–2010.
- Deer, D. M., Lampel, K. A., & Gonzalez-Escalona, N. (2010). A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse-transcription PCR assays. *Letters in Applied Microbiology*, 50, 366–372.
- Eischeid, A. (2016). Development and evaluation of a real-time PCR assay for detection of lobster, a crustacean shellfish allergen. *Food Control*, 59, 393–399.
- Eischeid, A., Kim, B.-H., & Kasko, S. (2013). Two quantitative real-time PCR assays for the detection of penaeid shrimp and blue crab, crustacean shellfish allergens. *Journal of Agricultural and Food Chemistry*, 61, 5669–5674.
- Eischeid, Anne C., Stadig, Sarah R., Handy, Sara M., Fry, Frederick S., & Deeds, Jonathan D. (2016). Optimization and Evaluation of a Method for the Generation of DNA Barcodes for the Identification of Commercial Decapod Crustaceans. *LWT-Food Science and Technology*, 73, 357–367.
- ENGL (European Network of GMO Laboratories), JRC Technical Report, 2015. *Definition of Minimum Performance Requirements for Analytical Methods of GMO Testing*.
- Hird, H., Chisholm, J., Sanchez, A., Hernandez, M., Goodier, R., Schneede, K., et al. (2006). Effect of heat and pressure processing on DNA fragmentation and implications for the detection of meat using a real-time polymerase chain reaction. *Food Additives and Contaminants*, 23(7), 645–650.
- Sakai, S., Adachi, R., Akiyama, H., & Teshima, R. (2013). Validation of quantitative and qualitative methods for detecting allergenic ingredients in processed foods in Japan. *Journal of Agricultural and Food Chemistry*, 61, 5675–5680.
- Sharma, G. M., Khuda, S. E., Parker, C. H., Eischeid, A. C., & Pereira, M. (2017). Detection of allergens in food: analytical methods. In U. G. Spizzirri, & G. Cirillo (Eds.). *Food safety: Innovative analytical tools for safety assessment* (pp. 65–122). Scrivener Publishing LLC.
- Sicherer, S. A., Munoz-Furlong, A., & Sampson, H. A. (2004). Prevalence of seafood allergy in the United States determined by a random telephone survey. *Journal of Allergy and Clinical Immunology*, 114, 159–165.
- Taguchi, H., Watanabe, S., Temmei, Y., Hirao, T., Akiyama, H., Sakai, S., et al. (2011). Differential detection of shrimp and crab for food labeling using polymerase chain reaction. *Journal of Agricultural and Food Chemistry*, 59, 3510–3519.
- U.S. Food and Drug Administration (U.S. FDA), Office of Foods and Veterinary Medicine. (2015). *Guidelines for the Validation of Chemical Methods for the FDA FVM Program*. Available at: < <http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf> > .
- Wild, L. G., & Lehrer, S. B. (2005). Fish and shellfish allergy. *Current Allergy and Asthma Reports*, 5, 74–79.

SUPPLEMENTARY MATERIAL

Figure S1: Assay Design Screenshot: king crabs and swimming crabs. Shows complete sequences for all primers and probe. In order from top to bottom, the crab species shown in the “Bind” column are: *Paralithodes brevipes*, *Paralithodes camtschaticus*, *Lithodes nintokuae*, *Scylla olivacea*, *Scylla serrata*, *Scylla paramosain*, *Scylla tranquebarica*, *Portunus trituberculatus*, and *Callinectes sapidus*. See Figure S3 for more detail on species.

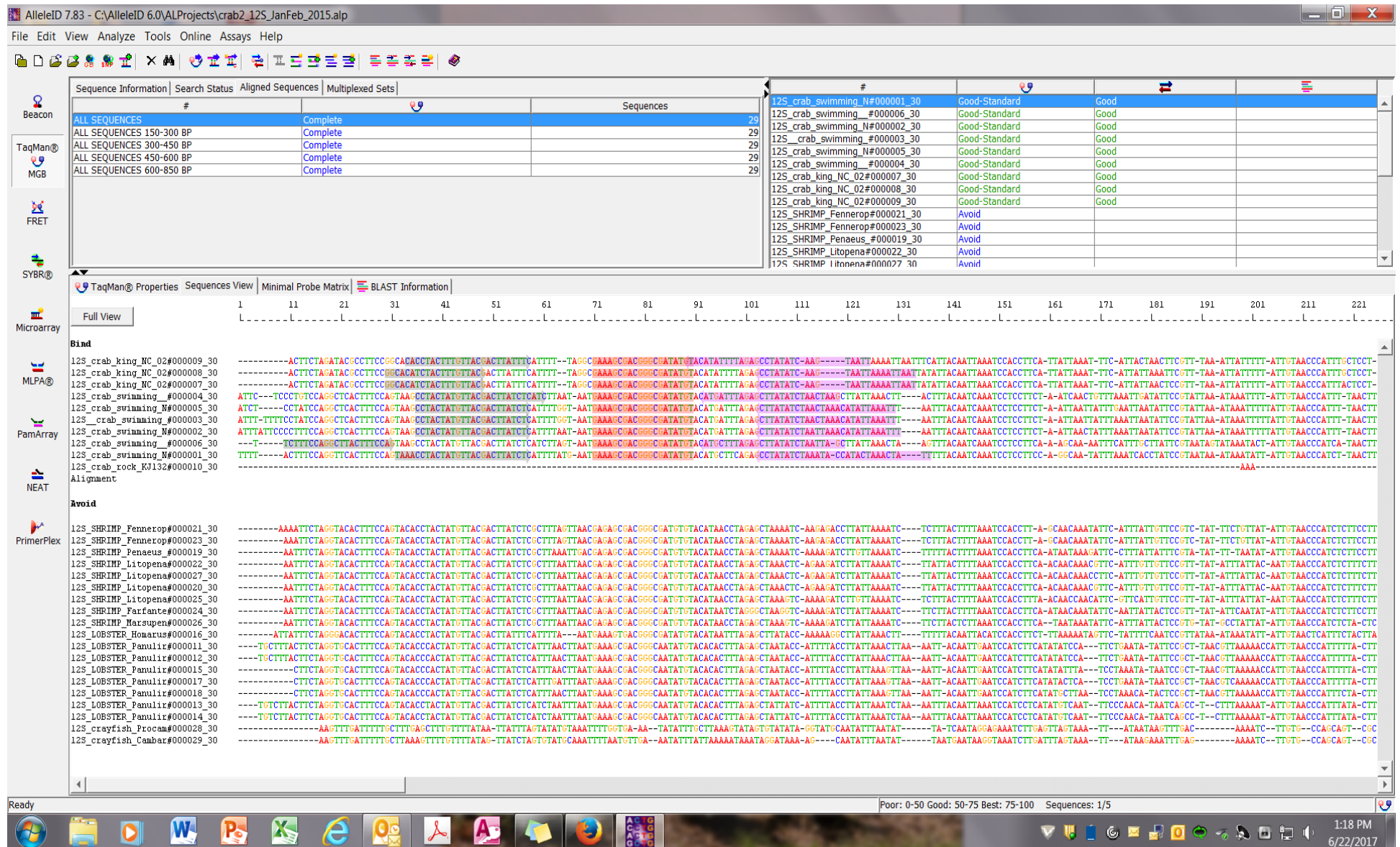


Figure S2: Assay Design Screenshot: rock crab. Shows complete sequences for all primers and probe. In order from top to bottom, the crab species shown in the “Bind” column are: *Paralithodes brevipes*, *Paralithodes camtschaticus*, *Lithodes nintokuae*, *Scylla olivacea*, *Scylla serrata*, *Scylla paramosain*, *Scylla tranquebarica*, *Portunus trituberculatus*, and *Callinectes sapidus*. See Figure S3 for more detail on species.

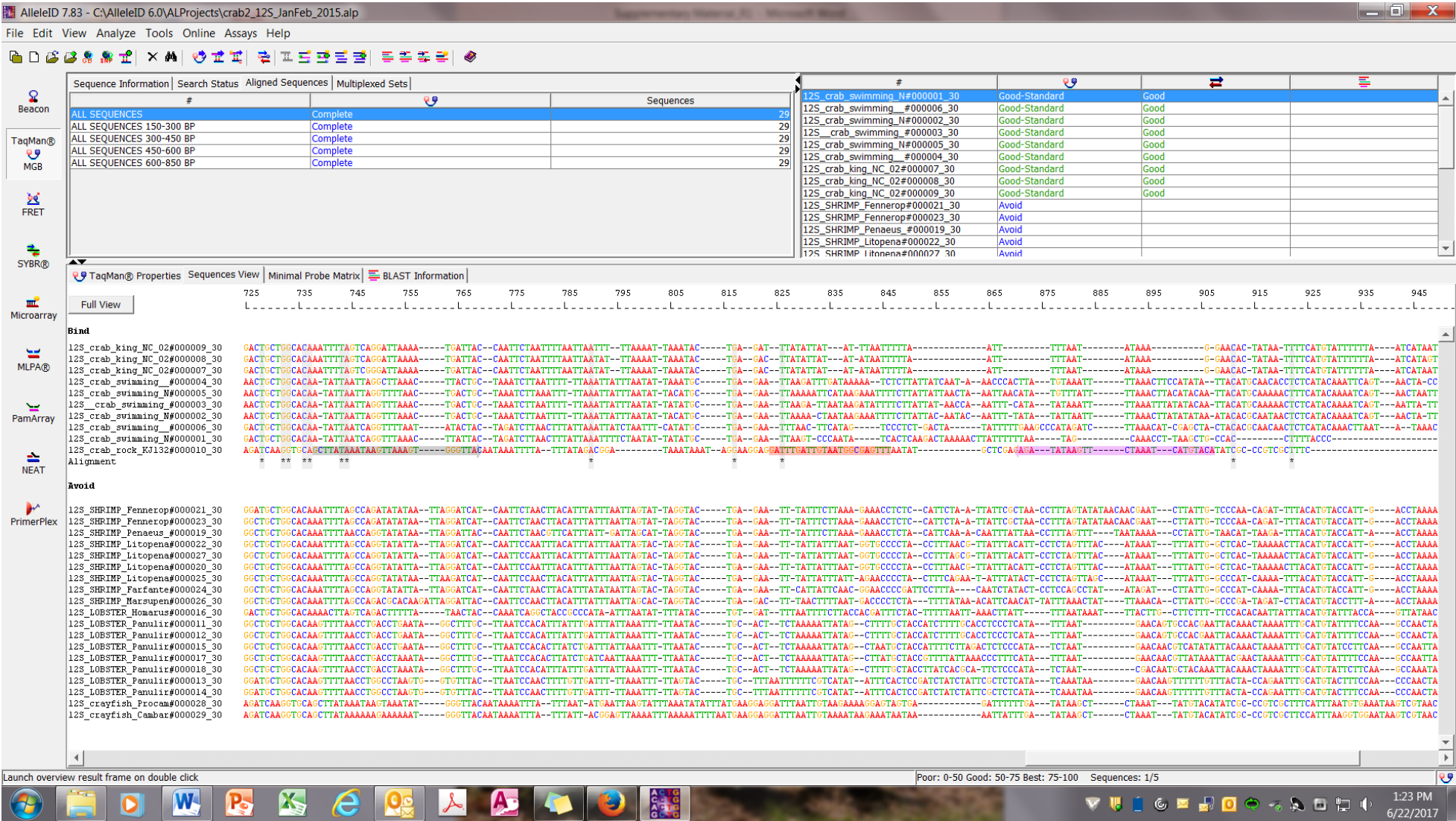


Figure S3: Species list exported from primer design software. Numbering in first column (000001, 000002, etc.) corresponds to numbering on left side of Figures S1 and S2 above. Names in definition column are gene_type of crab_accession number_scientific name. For example, the first item listed under “Bind” in Figures S1 and S2 is #000009=*000009,12S gene, king crab, accession number NC_021458, scientific name *Paralithodes brevipes*.

AlleleID 7.83 - C:\AlleleID 6.0\ALProjects\crab2_12S_JanFeb_2015.alp

File Edit View Analyze Tools Online Assays Help

Sequence Information | Search Status | Aligned Sequences | Multiplexed Sets

#	Definition	Length
*000001	12S_crab_swimming_NC_006281_Callinectes_sapideus	785
*000002	12S_crab_swimming_NC_012567_scylla_tranquebarica	869
*000003	12S_crab_swimming_NC_012572_scylla_paramosain	870
*000004	12S_crab_swimming_NC_012569_scylla_olivacea	852
*000005	12S_crab_swimming_NC_012565_scylla_serrata	874
*000006	12S_crab_swimming_NC_005037_Portunus_trituberculatus	840
*000007	12S_crab_king_NC_024202_Lithodes_nintokuuae	821
*000008	12S_crab_king_NC_020029_Paralithodes_camtschaticus	819
*000009	12S_crab_king_NC_021458_Paralithodes_brevipes	789
*000010	12S_crab_rock_KJ132446_Metacarcinus_magister_partial	454
*000011	12S_LOBSTER_Panulirus_stimpsoni_NC_014339	870
*000012	12S_LOBSTER_Panulirus_stimpsoni_GQ292768	870
*000013	12S_LOBSTER_Panulirus_japonicus_NC_004251	870
*000014	12S_LOBSTER_Panulirus_japonicus_AB071201	870
*000015	12S_LOBSTER_Panulirus_homarus_NC_016015	859
*000016	12S_LOBSTER_Homarus_americanus_NC_015607	843
*000017	12S_LOBSTER_Panulirus_ornatus_NC_014854	856
*000018	12S_LOBSTER_Panulirus_versicolor_NC_017868	862
*000019	12S_SHRIMP_Penaeus_monodon_AF217843	852
*000020	12S_SHRIMP_Litopenaeus_vannamei_NC_009626	856
*000021	12S_SHRIMP_Fenneropenaeus_chinensis_DQ518969	852
*000022	12S_SHRIMP_Litopenaeus_vannamei_EF584003	856
*000023	12S_SHRIMP_Fenneropenaeus_chinensis_DQ656600	855
*000024	12S_SHRIMP_Farfantepenaeus_californiensis_EU497054	852
*000025	12S_SHRIMP_Litopenaeus_stylirostris_EU517503	852
*000026	12S_SHRIMP_Marsupenaeus_japonicus_AP006346	853
*000027	12S_SHRIMP_Litopenaeus_vannamei_DQ534543	853
*000028	12S_crayfish_Procambarus_clarkii_NC_016926	786
*000029	12S_crayfish_Cambaroides_similis_NC_016925	787

Commercial Food Matrix Ingredients

Pork Dumplings

Filling [Pork, Leeks, Green Cabbage, Napa Cabbage, Pork Fat, Green Onions, Egg Whites, Concentrated Chicken Broth, Ginger, Sesame Oil, Corn Starch, Soybean Oil, Corn Maltodextrin, Salt, Garlic, Soy Sauce (Fermented Soybeans, Wheat And Salt), Mushroom Extract, Chili Paste (Red Chili Peppers, Distilled Vinegar, Salt, Xanthan Gum)], Dumpling Wrapper [Wheat Flour, Water, Salt, Sodium Tripolyphosphate], Sauce [Soy Sauce (Water, Wheat, Soybeans, Salt, Alcohol, Vinegar), Water, Rice Wine Vinegar, Sugar, Hot Chinese Mustard (Vinegar, Oriental Mustard Seed, Salt, Spices, Artificial Flavor, Capsicum, Turmeric {Color}), Ginger Puree (Ginger, Water), Soybean Oil, Xanthan Gum)].

Macaroni Salad

Elbow Macaroni (Semolina, Niacin, Ferrous Sulfate [Iron], Thiamin Mononitrate, Riboflavin, Folic Acid), Salad Dressing (Soybean Oil, Water, Corn Syrup, Distilled Vinegar, Modified Food Starch, Egg Yolks, Sugar, Salt, Spices, Paprika), Water, Sugar, Eggs, Green Peppers, Onion, Carrots, Mustard (Water, Distilled Vinegar, Mustard Seed, Salt, Mustard Bran, Turmeric, Natural Flavor, Paprika), Modified Cornstarch, Distilled Vinegar, Salt, Maltodextrin, Cultured Dextrose, Sodium Diacetate, Nisin Preparation (Nisin, Salt), Egg White Lysozyme.

Clam Juice

Clam juice [water, clam extract], salt