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### Introduction

The consumption of shrimp continuously increases worldwide due to its well-established health benefits. For the majority of world's population, the growing interest can be considered a nutritional advantage. However, for a small but rather significant group of food-allergic individuals, the consumption can pose a severe health problem.<sup>1</sup> Many cases of shellfish allergies have been frequently reported, being currently viewed as an emergent issue of public health.<sup>2,3</sup>

In the European Union (EU), food labeling regulations have been revised and labeling of several allergenic ingredients is now mandatory (Directives 2003/89/EC and 2007/68/EC).<sup>4</sup> But different countries mandate a different selection of allergens for food labeling. Despite this regulation, total avoidance might be difficult for the allergic consumer. Furthermore, little is known on threshold doses, *i.e.* the minimum amount of an allergenic food which is able to cause an allergic reaction. Hence, the sensitive and selective analytical methods for allergens are required to protect certain consumers.

The most frequently used analytical methods for allergen detection are either immunological based on antibodies or polymerase chain reactions (PCR).<sup>2</sup> Many disadvantages of current established methods for allergen analyses are discussed, such as cross-reactivity and potential false-negative results.<sup>5-8</sup> Recently, LC/MS technique using protease digestion

# Authentication of shrimp muscle in complex foodstuff by in-solution digestion and high-resolution mass spectrometry

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A method for shrimp muscle identification in complex foods is required to safeguard the shrimp-allergic population. This study described a method for authentication of shrimp in complex foodstuffs (fish balls) by liquid chromatography tandem QTOF mass spectrometry (UPLC-QTOF-MS). The proteins in shrimp muscle were extracted using a Tris–HCl solution and then digested using tryptic protease. The main allergen proteins, tropomyosin (TM) and arginine kinase (AK), were characterized using the 'bottom up' MS approach. After analysis of their peptide mass fingerprinting based on the UniProt database, two specific heat-stable peptides, ALSNAEGEVAALNR for TM and VSSTLSSLEGELK for AK, were screened as surrogate (signature) peptides. The detection limit, expressed as shrimp meat per kilogram of food, was 8 g kg<sup>-1</sup> (usage of TM) or 5 g kg<sup>-1</sup> (usage of AK). The developed method is suitable to screen potential addition of shrimp meat in foodstuffs by detection of allergen proteins.

was developed to characterize and identify proteins.<sup>9-12</sup> Different mass analyzer including triple quadrupole (QQQ), time-of-flight (TOF), orbitrap and ion trap (IT) are applied for the allergen test.<sup>13,14</sup> Because MS identification is a direct type of detection using different principles, it is expected to be an effective method for allergen confirmation. Noticeably, identifying and detecting allergens using MS systems, sample preparation is a critical step. Allergen proteins are usually extracted from food matrice and digested with enzymes generating peptides<sup>15</sup>

In this study, we aimed to analysis shrimp allergen protein in complex foodstuffs (fish balls) by liquid chromatography tandem QToF mass spectrometry (UPLC-QToF-MS). The allergen protein was prepared by in-solution digestion and (solid phase extraction) SPE clean-up. The main allergen protein in shrimp, tropomyosin (TM) or arginine kinase (AK) was tested by their optimized signature peptides.

## Experimental

#### Chemicals and materials

Ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>), dithiotheritol (DTT), iodoacetamide (IAA) and hydrochloric acid (HCl, 37%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and formic acid (FA) were purchased from Merck (Darmstadt, Germany). All the reagents used were analytical or HPLC grade. Sequencing grade modified trypsin was from Shanghai Yaxin Biotechnology Co., Ltd (Shanghai, China). All chemical agents were prepared using ultrapure water and without further purification. Ultrapure water was obtained by

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a Milli-Q Gradient A10 water purification system (Millipore, Bedford, MA, USA) during all the experiments.

#### Samples

The samples (fish balls) were purchased from local supermarket (TESCO, Hangzhou, China). Fish balls are mainly made from fish meat, starch, pork meat or shrimp meat. For validation of developed method, the home-made fish balls containing 10, 50, 100, 150, 200 g kg<sup>-1</sup> shrimp meat were prepared by mixture of fish meat (*Anguilla japonica*), starch (*Dioscorea esculenta* Burkill), pork meat (*Yorkshire*), shrimp meat (*Penaeus vannamei*) and water. The home-made fish balls are cooked in boiled water for 30 min. The samples were stored at -20 °C before experimental analysis.

#### Sample extraction and digestion

Allergen proteins were extracted from 5 g ground matrix in 15 mL Tris-HCl (200 mM, pH 9.2) with 2 M urea by shaking for 30 min. Then, the mixture was sonicated for 15 min at 4 °C to avoid carbamidomethylation with urea. The samples were centrifuged at 5000 g for 15 min at 10 °C. 100 µL supernatant, 100 µL 500 mM NH4HCO3 and 665 µL deionized water were mixed in an Eppendorf tube. 10 µL 50 mM DTT solution were added to the mixtures and reduced in 40 °C water bath for 30 min at this stage. In the next step an alkylation was performed by adding 10  $\mu$ L of 150 mM IAA in the dark for 30 min at room temperature. Immediately prior to the incubation, 100 µL of 500 mM NH<sub>4</sub>HCO<sub>3</sub> and 10 µL of 400 µg m L<sup>-1</sup> trypsin (freshly prepared) were added and incubated 6 h at 37 °C. The reaction was terminated by addition of 5 µL formic acid. The insoluble substances in tryptic hydrolysates were removed by centrifuging at 13 000g for 10 min. Before analysis of Q-TOF, the supernatant was further cleaned up by SPE.

For screening of the surrogated peptides for the allergens, 0.5 g shrimp muscle sample was directly selected, and prepared by the above method.

#### Peptides clean-up

SPE purification of digested proteins was performed on Oasis® HLB SPE columns (3 cm<sup>3</sup>/60 mg, 30  $\mu$ m). Cartridge was preconditioned with 1 mL MeOH followed by equilibration with 1 mL water of 0.1% formic acid. The digested samples were loaded on the column. The samples were washed with 1 mL of 20% MeOH in water. The analytes were eluted with 1 mL MeOH and then dried down under nitrogen. The dried samples were reconstituted into 1 mL of 2% acetonitrile in water with 0.1% formic acid. The mixture was analyzed by UPLC-QToF-MS after passing through a 0.22  $\mu$ m nylon filter (Agela Technologies).

#### Instrument conditions

Tryptic hydrolysates were separated using an ACQUITY UPLC System equipped with ACQUITY UPLC binary solvent manager, sample manager, and column manager (Waters, Milford, MA, USA). Chromatographic separation was carried out on a narrow-bore Acquity UPLC BEH 300C<sub>18</sub> column (1.7  $\mu$ m, 2.1 mm  $\times$  100 mm) maintained at 35 °C, equipped with a guard column of the same material (Waters, Milford, MA, USA). The 0.1% FA aqueous solution (solvent A) and 0.1% FA ACN solution (solvent B) were used for the mobile phases. Gradient elution was: 2% B to 40% B for 20 min; 100% B for 1 min; re-equilibration at the initial conditions for 2 min. The flow rate for separations was maintained at 0.3 mL min<sup>-1</sup> and a 10.0  $\mu$ L injection volume was used for all standards and samples.

Quadruple time-of-flight tandem mass spectrometry (QToF-MS) detection was performed on a Synapt G2 HDMS equipped with an electrospray ion (ESI) source (Waters). All data were acquired in the electrospray positive ion (ESI<sup>+</sup>) mode with MS<sup>E</sup> mode. Details of TOF conditions were as follows: capillary voltage, 3 kV; sampling cone voltage, 25 V; extraction cone voltage, 4 V; source temperature, 100 °C; desolvation temperature, 400 °C; cone gas flow, 30 L h<sup>-1</sup>; desolvation gas flow, 800 L h<sup>-1</sup>; ramp trap collision energy, 15–35 V; and lockspray reference compound, leucine-enkephalin (m/z 556.2771 Da).

#### Allergen database and data processing

The MS<sup>E</sup> data were searched against the shrimp allergen database containing TM and AK of Litopenaeus vannamei (whiteleg shrimp) (downloaded from http://www.uniprot.org/ on Jan. 10, 2015) using the IDENTITY<sup>E</sup> search algorithm within the ProteinLynx Global Server v. 2.5 (PLGS 2.5; Waters, U.K.). Search parameters included the "automatic" setting for mass accuracy (50 ppm for precursor ions and 0.1 Da for product ions), a minimum of one peptide match per protein, a minimum of three consecutive product ion matches per peptide, and a minimum of seven total product ion matches per protein. The maximum false positive rate (FPR) against the randomized forward database was set to 4%. Only one missed tryptic cleavage site was allowed during the search. Modifications included the following: fixed, carbamidomethylation of Cys; variable, deamidation of Asn and Gln, oxidation of Met, and dehydration of Ser and Thr.

#### Method validation

Method validation was performed based on the international conference on harmonization (ICH) guidelines<sup>16</sup> for validation of bio-analytical procedures. For linearity measurements, fish balls containing shrimp meat (*Penaeus vannamei*) with six different concentrations (10, 50, 100, 150, 200 g kg<sup>-1</sup>) along with blank samples were performed for calibration curves over 3 days.

Method acceptance criteria states that the precision of the calibration curve and QC samples (containing 1.5% shrimp meat) are considered to be acceptable if RSD  $\leq$  15% for intra and inter day precision. Furthermore, the accuracy compared with the nominal value needs to be no more than 15%. Finally, the calibration curves must meet the above criteria and have a correlation coefficient *r* of at least 0.99.

#### Analysis of tryptic peptides

Selection of suitable signature peptides for accurate quantitation of targeted protein is a crucial challenge for developing LC-MS/MS approach. Bioinformatics tool is usually adopted to assist the computational prediction of tryptic products. The theoretical tryptic cleavage peptides of allergen proteins were obtained by computational prediction by Waters Biolynx softwares and online PeptideMass tools provided by UniProt (http:// web.expasy.org/peptide\_mass).

For further confirmation of tryptic peptides, UPLC-QToF-MS was applied for comparing the endogenous and theoretical peptides from tryptic proteins. These peptides were identified detected in tryptic shrimp muscles after comparing the acquired data and sequence database search (Table 1). The coverage ratios of the searched peptides to targeted protein were all more than 50%. Theoretically, tryptic peptides numbers of TM and AK from *Litopenaeus vannamei* (whiteleg shrimp) were 32 and 29. But, actual detected peptides in MS showed in Table 1 were less than those. Not all the obtained peptides can be used as the surrogate (signature) peptides. The candidate peptides were selected based on several critical factors such as specificity of amino acid sequences, reproducibility in sample preparation, intensity of their MS signal.<sup>17</sup>

#### Selection of signature peptides

Applied criteria for signature peptides are the absence of cysteine and methionine, peptide size between seven and 20 amino acids, and no modification of amino acids. Furthermore, the peptides with more amino acids (>14) may be not used due to their expensive synthesize and unfavorable LC properties.<sup>17,18</sup> Therefore, we selected the peptide VSSTLSSLEGELK, LIDDHFLFK, LTSAVNEIEK and TFLVWVNEEDHLR for AK, and IQLLEEDLER, ALSNAEGEVAALNR, IVELEEELR, LAEASQAA-DESER and EVDRLEDELVNEK for TM.

With the aim to screening these peptides with the property of easy tryptic digestion and high MS intensity, we reduced the digestion time to 2 h and investigated the MS intensity of these peptides. As showed in Fig. 1, different intensities of peptides were obtained. The signature peptides, IQLLEEDLER, IVE-LEEELR and ALSNAEGEVAALNR for TM and VSSTLSSLEGELK and LIDDHFLFK for AK were selected. The difference of tryptic hydrolysis degree within peptides is hard to explain. Although disulfide bonds of proteins were sheared by DTT and IAA in the pretreatment process, the secondary structure main composed of hydrogen bond and van der Waals forces still existed.

For further optimization of signature peptides, the sequences of TM and AK in some crabs and shrimps were aligned (Fig. 2). We selected the peptides, ALSNAEGEVAALNR for TM and VSSTLSSLEGELK for AK, which are not presented in most crabs. In addition, the intensities of the two peptides were not significantly changed after treatment with boiled water for 30 min (n = 8, P < 0.05).

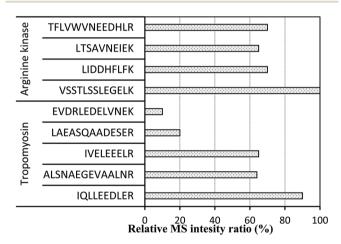


Fig. 1 he intensity of peptides obtained by 2 h digestion.

|                    | Peptides (*recommended |                                  | Precursor | Mass error        |        |  |
|--------------------|------------------------|----------------------------------|-----------|-------------------|--------|--|
| Protein            | signature peptides)    | Matched products string          | intensity | Precursor $(m/z)$ | (ppm)  |  |
| B4YAH6 LITVA Lit v | IQLLEEDLER             | b2b2b3y1y3y5y6y7y8y9y10          | 12 938    | 629.3392          | 3.5958 |  |
| 1 tropomyosin      | *ALSNAEGEVAALNR        | b9b14y5y6y7y8y9y10y14            | 8017      | 707.8684          | 2.0720 |  |
|                    | IVELEEELR              | y2y4y6y7y7y8y9                   | 6999      | 565.3094          | 1.8585 |  |
|                    | LAEASQAADESER          | y4y5y7y9y10y13                   | 6492      | 688.8181          | 2.8633 |  |
|                    | SITDELDQTFSELSGY       | b10b13b16y10y11y16               | 5227      | 902.9098          | 3.1328 |  |
|                    | LAMVEADLER             | y5y6y7y8                         | 1250      | 573.7949          | 5.1705 |  |
|                    | EVDRLEDELVNEK          | b7b8y4y5y7y11y12                 | 1040      | 794.3971          | 5.6077 |  |
| Q004B5 LITVA       | LGFLTFCPTNLGTTVR       | b16y5y7y9y10y11y12y16            | 23 790    | 898.9724          | 3.3503 |  |
| arginine kinase    | *VSSTLSSLEGELK         | b5b6b8b13b13y4y6y8y9y10y11y12y13 | 16 996    | 675.3613          | 5.8746 |  |
|                    | DFGDVNSFVNVDPEGK       | b11y4y5y7y10y16                  | 9333      | 869.8987          | 3.5018 |  |
|                    | *LIDDHFLFK             | b6y5y6y7y9                       | 3888      | 574.3119          | 4.2465 |  |
|                    | LTSAVNEIEK             | y5y6y7y9y10                      | 3506      | 552.3025          | 4.9357 |  |
|                    | EMQDGILELIK            | y5y7y8y9                         | 2755      | 644.8461          | 5.3712 |  |
|                    | TFLVWVNEEDHLR          | y3y7y8y9y10y11y13                | 2641      | 829.4190          | 3.0708 |  |
|                    | FLQAANACR              | b5y1y5y6y7                       | 2093      | 1050.5166         | 1.3153 |  |
|                    | GEHTEAEGGIYDISNK       | b8b9b10y4y5y9                    | 1279      | 860.3935          | 3.2045 |  |

Table 1 Identified peptides by in-matrix digestion and UPLC-QTOF-MS

#### Align of selected proteins for tropomyosir

CLUSTAL O(1.2.2) multiple sequence alignment

TR|B4YAH6|B4YAH6\_LITVA

| 60  |        |              |  |     |  |  |
|---|--------|--------------|--|-----|--|--|
|   |        | D3XNS0_FENME | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV | 60  |  |  |
| SP  | P86704 | TPM_PANBO    | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVFGLQKKLQQLENDLDSV | 60  |  |  |
|   |        | TPM_CHAFE    | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANLRAEKTEEEIRATQKKMQQVENELDQA | 60  |  |  |
| SP  | A1KYZ2 | TPM_PENMO    | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV | 60  |  |  |
|   |        | TPM_METEN    | MKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV           | 50  |  |  |
| TR  | E7CGC1 | E7CGC1_PENMO | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV |     |  |  |
| TR  | D7F1J4 | D7F1J4_CRACN | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVFSLQKRMQQLENDLDSV | 60  |  |  |
|   |        |              | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV |     |  |  |
|   |        |              | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKTEEEIRATQKKMQQVENELDQA |     |  |  |
| TR  | A5D6H8 | A5D6H8_LIMPO | IKNKMQAMKLDKENACDRADIAEQQSRDANARADKAEEEVRSLQKKIQQIENELDQV    | 57  |  |  |
|   |        |              | MDAIKKKMQAMKLEKDNAMDRANTLEQQNKEANLRAEKTEEEIRATQKKMQQVENELDQA |     |  |  |
|   |        |              | MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVFSLQKRMQQLENDLDSV |     |  |  |
|   |        | M1H607_PORPE | MDAIKKKMQAMKLEKDDAMDRADTLEQQNKEANIRAEKABEEVHNLQKRMQQLENDL    | DQV |  |  |
| MDAIKKKMQAMKLEKDNAMDRADTLEQQNKEANNRAEKSEEEVHNLQKRMQQLENDLDQV 60 |        |              |  |     |  |  |
|   |        |              | **::*:.* *::: * *.:::* :*:*:::*: **: * :*:*:                 |     |  |  |
|   |        |              |  |     |  |  |

| TR   | B4YAH6 | B4YAH6 LITVA | QESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|--|--------|--------------|--|--|--|--|
| SP   | P86704 | TPM_PANBO    | QEALLKANQHLEEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        | TPM_CHAFE    | QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLPEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        | TPM_PENMO    | QESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
| SP   | Q25456 | TPM_METEN    | QESLLKANNQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 110 |  |  |  |
| TR   | E7CGC1 | E7CGC1_PENMO |  |  |  |  |
|  |        | D7F1J4_CRACN |  |  |  |  |
|  |        |              | QESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        |              | QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        |              | QEQLVTANAKLEEKDKALQNAEGEVAALNRRVQLLEEDLERSEERLKIATQKLEEATQLA 117 |  |  |  |
| TR   | A7L5V3 | A7L5V3_PORTR | QEQLSAANTKLDEKEKALQNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        |              | QEALLKANQHLEEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        |              | QESLLKANTQLEEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
| TR   | D3XNS0 | D3XNS0_FENME | QESLLKANIQLVEKDKALSNAEGEVAALNRRIQLLEEDLERSEERLNTATTKLAEASQAA 120 |  |  |  |
|  |        |              | .* * *:* **:**: ********************                             |  |  |  |
| Align of selected proteins for arginine kinase |        |              |  |  |  |  |
|  |        |              | -  |  |  |  |

CLUSTAL O(1.2.2) multiple sequence alignment

| CLUSTAL 0(1.2.2  | ) multiple s  |  |   |
|--|---|--|---|
| SP Q95V58 KARG_AR<br>SP P51545 KARG_PE   | TSF   | MVDAGTLEKLEAGFQKLQAATDCKSLVKKYLTREVFDQLKTLKTS-LGATLLDVIQSGVE<br>- VDAAVLEKLOAGFKKLEAATDCKSLLKKYLSKDIFDKLKGOKTS-LGATLLDVIOSGVE  | 59<br>58  |
| SP Q9NH48 KARG ERISI   |   | MADAATIAKLDEGFKKLEAATDCKSLLKKYLTKDVFEQLKAKKTK-LGATLLDVIQSGVE   | 59  |
| SP Q9NH49 KARG_CALSI<br>SP P51541 KARG LIMPO   |   | MADAATIAKLEEGFKKLEAATDCKSLLKKYLTKSVFDQLKDKKTS-LGATLLDVIQSGVE<br>MVDOATLDKLEAGFKKLOEASDCKSLLKKHLTKDVFDSIKNKKTG-MGATLLDVIOSGVE   | 59<br>59  |
| SP 09U9J4 KARG CARMA   |   | MADAATITKLEEGFKKLEAATDCKSLLKKYLTKSVFDQLKAKKTS-LGATLLDVIQSGVE   | 59  |
| SP Q9GYX1 KARG_PACMR<br>SP C7E3T4 KARG_PENMO   |   | MADAATISKLEEGFKKLQGATDCKSLLKKYLTKDVFDQLKAKKTS-LGATLLDVIQSGVE   | 59<br>59  |
| TR E7CGC2 E7CGC2 PENMO   |   | MADAAVIEKLEAGFKKLEAATDCKSLLKKYLSKAVFDQLKEKKTS-LGATLLDVIQSGVE<br>MADAAVIEKLEAGFKKLEAATDCKSLLKKYLSKAVFDQLKEKKTS-LGATLLDVIQSGVE   | 59  |
| TR D7F1J5 D7F1J5 CRACN   |   | MVDAEVLEKLEAGYKKLEAATDCKSLLKKYLTKEVFDELKTKKTA-LGATLLDVIQSGVE<br>MADAAVIEKLEAGFKKLEAATDCKSLLKKYLTKEVFDKLKDKKTS-LGATLLDVIQSGVE   | 59<br>59  |
| TR   B1PVZ9   B1PVZ9_METEN<br>TR   Q004B5   Q004B5_LITVA   |   | MADAAVIEKLEAGFKKLEAATDCKSLLKKYLTKEVFDKLKDKRTS-LGATLLDVIOSGVE   | 59  |
| TR A0A088FIL9 A0A088FIL9 HALRR   |   | MVDTEVLEKLEAGFKKLQDAKDCKSLLKKYLTQEVFDELKTKKTS-LGATLLDVIQSGVE   | 59<br>59  |
| TR   G3D692   G3D692   | PENCH<br>PORTR  | MADAAVIEKLEAGFKKLEAATDCKSLLKKYLTKAVFDQLKDKKTS-LGATLLDVIQSGVE<br>MADAATIAKLEEGFKKLEAATDCKSLLKKYLTKSVFDQLKDKKTD-LGATLLDVIQSGVE   | 59  |
| TR   H6UKS0   H6UKS0<br>TR   C3VUU0   C3VUU0   | SCYPA   | MADAATIAKLEEGFKKLEAATDCKSLLKKYLTKSVFDQLKGKKTS-LGATLLDVIQSGVE<br>MADAAVIEKLEAGFKKLEAATDCKSLLKKYLTKEVFDKLKDKKTS-LGATLLDVIQSGVE   | 59<br>59  |
| TR[C3V000]C3V000_  | FENME   |  |   |
| SP Q95V58 KARG_AR<br>SP P51545 KARG_PE   |   | VISTRVRCGRSLQGYPFNPCLTEAQYKEMEDKVSSTLNGLDGELKGTFYPLTGMAKEVQQ<br>VISTRVRCGRSMEGYPFNPCLTEAQYKEMQQKVSSTLSSLEGELKGTYFPLTGMSKEVQQ   | 179<br>178  |
| SP Q9NH48 KARG ER  | ISI   | VISTRVRCGRSMEGYPFNPCLTEAQYKEMESKVSSTLSNLEGELKGTYFPLTGMTKEVQQ   | 179   |
| SP Q9NH49 KARG_CA<br>SP P51541 KARG_LI   |   | VISTRVRCGRSMEGYPFNPCLTEAQYKEMESKVSSTLSNLEGELKGTYFPLTGMTKEVQQ<br>IISTRVRCGRSLQGYPFNPCLTAEQYKEMEEKVSSTLSSMEDELKGTYYPLTGMSKATQQ   | 179<br>179  |
| SP Q9U9J4 KARG CA  | RMA   | VISTRVRCGRSMEGYPFNPCLTEAOYKEMESKVSSTLSNLEGELKGTYHALTGMTKDVOO   | 179   |
| SP Q9GYX1 KARG_PA<br>SP C7E3T4 KARG_PE   | CMR<br>NMO  | VISTRVRCGRSMEGYPPNPCLTEAQYKEMBAKVFSTLSSLEGELKGSFYPLTGMAKDVQQ<br>VISTRVRCGRSMEGYPPNPCLTEAQYKEMBAKVSSTLSSLEGELKGTYYPLTGMSKEVQQ   | 179<br>179  |
| TR E7CGC2 E7CGC2   | PENMO   | VISTRVRCGRSMEGYPFNPCLTEAQYKEMBAKVSSTLSSLEGELKGTYYPLTGMSKEVQQ   | 179   |
| TR   A0A097KVJ7   A0A<br>TR   D7F1J5   D7F1J5  | 097KVJ7_METMG   | SMEGYPFNPCLTEAQYKEMESKVSSTLSNLEGELKGTYHPLTGMTKDVQQ<br>VVSTRVRCGRSMEGYPFNPCLTEAQYKEMESKVSSTLSSLEGELKGTYYPLTGMSKDVQQ   | 50<br>179   |
| TR B1PVZ9 B1PVZ9   | METEN   | VISTRVRCGRSMQGYPFNPCLTESQYKEMBAKVSSTLSSLEGELKGTYYPLTGMSKEVQQ   | 179   |
| TR   A0A097KVS3   A0A<br>TR   Q004B5   Q004B5_   | 097KVS3_UCACR   | SMEGYPFNPCLTEAQYKEMEEKISSTLSNLEGELKGTYYPLTGMTKEVQQ<br>VISTRVRCGRSLQGYPFNPCLTESQYKEMEAKVSSTLSSLEGELKGTYYPLTGMSKEVQQ   | 50<br>179   |
| TR A0A088FIL9 A0A  | 088FIL9_HALRR   | VVSTRVRCGRSMEGYPFNPCLTEDQYREMEEKVSSTLSNLEGELKGTYYPLTGMSKEVQQ   | 179   |
| TR Q4KY22 Q4KY22<br>TR G3D692 G3D692   | FENCH   | VISTRVRCGRSMEGYPFNPCLTEDQYKEMESKVSSTLSSLEGELKGTYYPLTGMGKEVQQ<br>VISTRVRCGRSMEGYPFNPCLTEAQYKEMESKVSSTLSSLEGELKGTYFPLTGMTKEVQQ   | 179<br>179  |
| TR A0A097KVD1 A0A  | 097KVD1_ATEIN   | SMEGYPFNPCLTEAQYKEMESKVSSTLSGLEGELKGSYYPLTGMTKEVQQ   | 50  |
| TR   A0A097KVK5   A0A<br>TR   H6UKS0   H6UKS0_   | 097KVK5 PORPE   | SMEGYPFNPCLTEAQYKEMESKVSSTLSNLEGELKGTYFPLTGMTKEVQQ<br>VISTRVRCGRSMEGYPFNPCLTEAQYKEMESKVSSTLSNLEGELKGTYYPLTGMTKDVQQ   | 50<br>179   |
| TR G3C6N4 G3C6N4   | AUSED   | VISTRVRCGRSMEGYPFNPCLTEAQYKEMEDKVSSTLSGLEGELKGTFYPLTGMSKEVQQ   | 76  |
| TR A0A097KVQ6 A0A<br>TR C3VUU0 C3VUU0  | 097KVQ6_RANRA   | SMEGYPFNPCLTEAHYKEMESKVSSTLSGLEGELKGTYYPLTGNTKDVQQ<br>VISTRVRCGRSMOGYPFNPCLTESOYKEMEAKVSSTLSSLEGELKGTYYPLTGMSKEVOO   | 50<br>179   |
|  |   |  |   |
| Entry (tropomyosin)<br>B4YAH6  | Status<br>unreviewed  | Organism<br>Litopenaeus vannamei (Whiteleg shrimp) (Penaeus vannamei)  | Length<br>284   |
| P86704   | reviewed  | Pandalus borealis (Northern red shrimp)  | 284   |
| Q9N2R3   | reviewed  | Charybdis feriata (Crucifix crab) (Cancer feriatus)  | 264   |
| A1KYZ2   | reviewed  | Penaeus monodon (Giant tiger prawn)  | 284   |
| Q25456<br>E7CGC1   | reviewed<br>unreviewed  | Metapenaeus ensis (Greasyback shrimp) (Penaeus ensis)<br>Penaeus monodon (Giant tiger prawn)   | 274<br>284  |
| D7F1J4   | unreviewed  | Crangon crangon (Brown shrimp)   | 284   |
| D2KMW0   | unreviewed  | Fenneropenaeus chinensis (Fleshy prawn) (Penaeus chinensis)  | 284   |
| A4URH3   | unreviewed  | Eriocheir sinensis (Chinese mitten crab)   | 284   |
|  | uproviewed  | Linuulus polyphoneus (Atlantic horrachoa grab)   | 291   |
| A5D6H8   | unreviewed<br>unreviewed  | Limulus polyphemus (Atlantic horseshoe crab)   | 281<br>284  |
| A5D6H8<br>A7L5V3<br>V5NBV4   | unreviewed<br>unreviewed  | Limulus polyphemus (Atlantic horseshoe crab)<br>Portunus trituberculatus (Swimming crab) (Neptunus trituberculatus)<br>Macrobrachium rosenbergii (Giant fresh water pravm)   | 284<br>284  |
| A5D6H8<br>A7L5V3<br>V5NBV4<br>M1H607   | unreviewed<br>unreviewed<br>unreviewed  | Limulus polyphemus (Atlantic horseshoe crab)<br>Portunus trituberculatus (Swimming crab) (Neptunus trituberculatus)<br>Macrobrachium rosenbergii (Giant fresh water prawn)<br>Portunus pelagicus (Blue swimmer crab)   | 284<br>284<br>284   |
| A5D6H8<br>A7L5V3<br>V5NBV4   | unreviewed<br>unreviewed  | Limulus polyphemus (Atlantic horseshoe crab)<br>Portunus trituberculatus (Swimming crab) (Neptunus trituberculatus)<br>Macrobrachium rosenbergii (Giant fresh water pravm)   | 284<br>284  |
| A5D6H8<br>A7L5V3<br>VSNBV4<br>M1H607<br>D3XNS0   | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limulas polyphemus (Atlantic horeschoe crab)<br>Portums tribucculatus (Sviminue crah) (Neptuma tribucculatus)<br>Macrobrachium rosenbergii (Giant freich water pravn)<br>Portuma pologicas (Blue svimmer crab)<br>Fenneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)  | 284<br>284<br>284<br>284  |
| A5D6H8<br>A7L5V3<br>V5NBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase   | unreviewed<br>unreviewed<br>unreviewed<br>) Status  | Limulae polyphemus (Atlantic horeschoe erab)<br>Portums tribucarduna (Swimmigne erab) (Ortpannet tribuerulatus)<br>Macrobrachian resonbergii (Giant fieth water pravn)<br>Portuma pelagicae (Idle swimmer erab)<br>Fenneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Organism   | 284<br>284<br>284<br>284<br>284   |
| ASD6H8<br>A7L5V3<br>VSNBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58   | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) Status<br>reviewed  | Limulae polyhemus (Atlantic horeschoe erab)<br>Portunse trihoeraulans (Swimming erah) (Neptams trihoeraulans)<br>Macrobrachian rosenbergii (Giant fieth water pravn)<br>Porman pelagicas (Blue swimmer erab)<br>Fenneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Penneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Artemia franciscana (Brine shrimp) (Artemia sanfranciscana)  | 284<br>284<br>284<br>284<br>Length<br>356   |
| A5D6H8<br>A7L5V3<br>V5NBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase   | unreviewed<br>unreviewed<br>unreviewed<br>) Status  | Limulae polyphemus (Atlantic horeschoe erab)<br>Portums tribucarduna (Swimmigne erab) (Ortpannet tribuerulatus)<br>Macrobrachian resonbergii (Giant fieth water pravn)<br>Portuma pelagicae (Idle swimmer erab)<br>Fenneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Organism   | 284<br>284<br>284<br>284<br>284   |
| ASD6H8<br>A7L5V3<br>VSNBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58   | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) Status<br>reviewed  | Limulae polyhemus (Atlantic horeschoe erab)<br>Portunse trihoeraulans (Swimming erah) (Neptams trihoeraulans)<br>Macrobrachian rosenbergii (Giant fieth water pravn)<br>Porman pelagicas (Blue swimmer erab)<br>Fenneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Penneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Artemia franciscana (Brine shrimp) (Artemia sanfranciscana)  | 284<br>284<br>284<br>284<br>Length<br>356   |
| ASD6H8<br>A7L5V3<br>VSNBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545   | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) Status<br>reviewed<br>reviewed  | Limulae polyhemus (Atlantic horeschoe erab)<br>Portums triholeculauta (Swimmige erah) (Neptums triholeculauta)<br>Macrobrachiun rosenbergii (Giant fiesh water pravn)<br>Portums pelagicus (Blue swimmer erab)<br>Fenneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artenia sanfranciscana)<br>Penaeus japonicus (Kuruma pravn) (Marsupenaeus japonicus)  | 284<br>284<br>284<br>284<br>284<br>Length<br>356<br>355   |
| ASD6H8<br>A7L5V3<br>V5NBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9NH48   | unreviewed<br>unreviewed<br>unreviewed<br>) <u>Status</u><br>reviewed<br>reviewed<br>reviewed   | Limita polyhemus (Atlantic horeschoe erab)<br>Portunas tribuceulanta (Swimming erab) (Neptanus tribuerculatus)<br>Macrobrachna rosenbergii (Giant freih water pravn)<br>Permeroponaesa mergaiestis (Banasa pravn) (Penaesa mergaiensis)<br>Corganism<br>Artenia franciscoma (Brine shrimp) (Artenia sanfranciscoma)<br>Penaesa japonicus (Kuruma pernov) (Marsapenaesa japonicus)<br>Eriocheir sinensis (Chinese mitten erab)  | 284<br>284<br>284<br>284<br>284<br>Length<br>356<br>355<br>357  |
| ASD6H8<br>A7L5V3<br>VSNBV4<br>W11H607<br>D3XN80<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9NIH48<br>Q9NIH49<br>P51541  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) <u>Status</u><br>reviewed<br>reviewed<br>reviewed   | Limilae polyhemus (Allantic horeschoe enb)<br>Portums tribucaidan (Swimming enb) (Argunat Kithoreulatas)<br>Macrobrachian rosenbergii (Giant fieth water pravn)<br>Portums pologicae (UBa weinmer enb)<br>Fenneropenaeus merguiensis (Banua pravn) (Penaeus merguiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artenia sanfranciscana)<br>Penaeus japonicus (Kuruma pravn) (Marsupenaeus japonicus)<br>Eriocheir sinsmis (Chinese milten enb)<br>Calimeetes sapidus (Blue enb)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357  |
| ASD6H8<br>A7L5V3<br>VSNBV4<br>M1H607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9NH48<br>Q9NH49   | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed  | Limulua polyphemus (Allantic horeschoe erab)<br>Portums tribuceuladus (Swimming erab) (Neptums tribuceulatus)<br>Macrobrachiun rosenbergii (Giant fieth water pravn)<br>Portums pelagicus (Blue swimmer erab)<br>Fenneropenaeus merguiensis (Banana pravn) (Penaeus merguiensis)<br>Organism<br>Artemila franciscana (Brine shrimp) (Artemia sanfranciscana)<br>Penaeus japonicus (Kuruma pravn) (Marsupenaeus japonicus)<br>Eriocheir sinensis (Chinese milten erab)<br>Callinettes sapidas (Blue erab)<br>Limulus polyphemus (Allantic horeschoe erab)<br>Carcinus maenas (Common shore erab) (Green erab)   | 284<br>284<br>284<br>284<br>284<br>356<br>355<br>355<br>357<br>357  |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M11607<br>D3XN80<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N148<br>Q9N149<br>P51541<br>Q9U314<br>Q9U314<br>Q9U314  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) <u>Status</u><br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed   | Limita polyphemus (Atlantic horeschoe erab)<br>Portumas tribuceulands (Swimming erab) (Oxpanus Hindoweulants)<br>Macrobrachna rosenbergii (Giant fresh water pravn)<br>Pormoroponaeaa mergaioestis (Banana pravn) (Poraeaa mergaioensis)<br>Pomoroponaeaa mergaioestis (Banana pravn) (Poraeaa mergaioensis)<br>Organism<br>Organism<br>Artenia franciscana (Brine shrimp) (Artenia sanfranciscana)<br>Ponaeaa japonicus (Kuruma pravno) (Marsupenaeas japonicus)<br>Deriocheir sinensis (Chinese mitten erab)<br>Calimeters sapidas (Blue erab)<br>Limulus polyphemus (Atlantic horeschoe erab)<br>Carcinos meanas (Common shore erab) (Creen erab)<br>Paolograpusa marmoratus (Macbiel rock erab) (Cancer marmoratus)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357  |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M11607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51541<br>Q9N1449<br>P51541<br>Q9U314<br>Q9U314<br>Q9U314  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) <u>Status</u><br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed   | Limilae polyhemus (Allantic horeschoe erab)<br>Portums tribucardands (Swimmigne erab) (Argennar krithervulatera)<br>Macrobrachian resource gravity (Banara eravy) (Penaeus merguiensis)<br>Penneropenaeus merguiensis (Banara pravy) (Penaeus merguiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artenia sanfranciscana)<br>Penaeus japonicus (Kuruma pravyn) (Marsupenaeus japonicus)<br>Eriocheri sinansis (Chinese mittea erab)<br>Callinectes sapidus (Blue erab)<br>Limulus polyhemus (Atlantic horeschoe erab)<br>Carcinus maenarus (Common shore erab) (Green erab)<br>Penaeus marmoratus (Mathelder ock erab) (Caneer marmoratus)<br>Penaeus monodor (Giant tiger prawn)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357   |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M116607<br>D3XN80<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N148<br>Q9N149<br>P51541<br>Q9U34<br>Q9GYX1<br>C7E374<br>E7CGC2  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed  | Limilae polyhemus (Allanic horeschoe erab)<br>Portums tribuerulands (Swimmigne erab) (Argennus rituberulatus)<br>Macrobrachian resonbergii (Giant fieth water pravn)<br>Portums pologicus (Blue swimmer erab)<br>Fenneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Ponaeus iaponicus (Kuruma pravn) (Marsupenaeus japonicus)<br>Britocheir sinensis (Chinese mitten erab)<br>Callineetes sapidas (Blue erab)<br>Limulus polyhemus (Atlantic horeschoe erab)<br>Carcinus maenzu (Common shore erab) (Green erab)<br>Penaeus morrotus (Omaton shore erab) (Green erab)<br>Penaeus monodon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>356   |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M11607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51541<br>Q9N1449<br>P51541<br>Q9U314<br>Q9U314<br>Q9U314  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>) <u>Status</u><br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed   | Limilae polyhemus (Allantic horeschoe erab)<br>Portums tribucardands (Swimmigne erab) (Argennar krithervulatera)<br>Macrobrachian resource gravity (Banara eravy) (Penaeus merguiensis)<br>Penneropenaeus merguiensis (Banara pravy) (Penaeus merguiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artenia sanfranciscana)<br>Penaeus japonicus (Kuruma pravyn) (Marsupenaeus japonicus)<br>Eriocheri sinansis (Chinese mittea erab)<br>Callinectes sapidus (Blue erab)<br>Limulus polyhemus (Atlantic horeschoe erab)<br>Carcinus maenarus (Common shore erab) (Green erab)<br>Penaeus marmoratus (Mathelder ock erab) (Caneer marmoratus)<br>Penaeus monodor (Giant tiger prawn)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357   |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M116607<br>D3XN80<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N148<br>Q9N149<br>P51541<br>Q9U34<br>Q9GYX1<br>C7E374<br>E7CGC2  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed  | Limilae polyhemus (Allanic horeschoe erab)<br>Portums tribuerulands (Swimmigne erab) (Argennus rituberulatus)<br>Macrobrachian resonbergii (Giant fieth water pravn)<br>Portums pologicus (Blue swimmer erab)<br>Fenneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Ponaeus iaponicus (Kuruma pravn) (Marsupenaeus japonicus)<br>Britocheir sinensis (Chinese mitten erab)<br>Callineetes sapidas (Blue erab)<br>Limulus polyhemus (Atlantic horeschoe erab)<br>Carcinus maenzu (Common shore erab) (Green erab)<br>Penaeus morrotus (Omaton shore erab) (Green erab)<br>Penaeus monodon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>356   |
| A 50648<br>A 71,5V3<br>VSNBV4<br>M116607<br>D3XN80<br>Entry (arginine kinase<br>Q93V58<br>P51545<br>Q9N148<br>Q9N149<br>P51541<br>Q9U3/4<br>Q9GYX1<br>C7E3T4<br>EFCGC2<br>A0A097KVJ7   | unreviewed<br>unreviewed<br>versiewed<br>versiewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed  | Limitae polyphemus (Atlantic horeschoe erab)<br>Portunus tribuceulutas (Swimming erab) (Neptanus tribucerulutas)<br>Macrobrachna rosenbergii (Giant firsh water pravn)<br>Pemeropanae adegices (Blue swimmer erab)<br>Pomeropanae unergelenisti (Banaus pravn) (Venocus merguiensis)<br>Organism<br>Artemia franciscoma (Brine shirup) (Artemia sanfranciscoma)<br>Penaeus japonicus (Kurum peruw) (Marsupeneous japonicus)<br>Eriocheir sinensis (Chinese milten erab)<br>Callineteris sapidus (Blue erab)<br>Limulus polyphemus (Atlantic horeschoe erab)<br>Caricinus maenza (Common shore erab) (Green erab)<br>Penaeus anondon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)<br>Metacarcinus mesister (Dungeness erab) (Cancer magister)   | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>210  |
| ASD648<br>A7L5V3<br>VSNBV4<br>MII607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51541<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>EFCGC2<br>A0A097KV17<br>D7F115<br>B1PV29  | unreviewed<br>unreviewed<br>variewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyhemus (Atlantic horeschoe erab)<br>Portums tribucculands (Swimmigne erab) (Aptennus tribucculants)<br>Macrobrachian resubergii (Gianti fieth water pravn)<br>Pornamus piedgeica (Blue swimmer erab)<br>Organism<br>Artenia franciscana (Brine shrimp) (Atrenais sanfranciscana)<br>Ponnesus japonicus (Kuruma pravn) (Marsupenneus japonicus)<br>Eriocheri situnsis (Chines mitten erab)<br>Callinectes sapidus (Blue erab)<br>Limulta polyhemus (Atlantic horoschoe erab)<br>Callinectes sapidus (Blue erab)<br>Limulta polyhemus (Atlantic horoschoe erab)<br>Carcinus maenars (Common shore erab) (Green erab)<br>Penaeus monodon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)<br>Matacarcinus megister (Dangeness erab) (Cancer magister)<br>Caragon erango (Brown shrimp)<br>Matapaneus ensis (Greavyback shrimp) (Penaeus ensis)  | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>210<br>356   |
| A 50648<br>A 71,5V3<br>VS/NBV4<br>M116607<br>D3XNS0<br>D3XNS0<br>P31545<br>Q95V58<br>P31545<br>Q9NH48<br>Q9NH48<br>Q9NH49<br>P51541<br>Q9U34<br>Q9U34<br>Q9U34<br>Q9U34<br>Q9U34<br>Q9U34<br>Q9U34<br>Q9U34<br>D7F155<br>B1PVZ9<br>A0A097KV75  | unreviewed<br>unreviewed<br>versiewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed   | Limilae polyhemus (Allanic horseshoe enb)<br>Portums tribuceulands (Swimmigne enb) (Argenus tribuerulatus)<br>Macrobrachian resubregii (Giant fieth water pravn)<br>Pornamus pelagicae (Idles weinnet enb)<br>Ponneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artennia sanfranciscana)<br>Penaeus iaponicus (Kunum pravn) (Marsupenaeus japonicus)<br>Eriocheir sinursis (Chiness mitten enb)<br>Calimetes sanjaha (Blue enb)<br>Limulus polyhemus (Atlantic horseshoe enb)<br>Carcinus meanas (Common shore enb) (Green enb)<br>Denaeus monodon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)<br>Metacaercinus meafare (Daneses enb) (Cancer magister)<br>Caragen erangon (Brown shrimp)<br>Metaquenaeus envis (Greatyback shrimp) (Penaeus envis)<br>Metaquenaeus envis (Greatyback shrimp) (Penaeus envis)<br>Lica erasis (Fiddler enab)  | 284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357  |
| A5D648<br>A7L5V3<br>VSNBV4<br>M111607<br>D3XN80<br>P31545<br>Q9X148<br>Q9X148<br>Q9X148<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>D51541<br>Q9QYX1<br>C7E374<br>C7E374<br>D7F105<br>B1PV29<br>A0A097KV33<br>Q804B5  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyphemus (Atlantic horeschoe erab)<br>Portunas tribuceulands (Swimming erab) (Oxpannis tribuceulatus)<br>Macrobrachna rosenbergii (Giant fichs water pravn)<br>Pornorponaeta mergaietusi (Banasa pravn) (Peraecas mergaietusi)<br>Organism<br>Organism<br>Artenia franciscoma (Brine shrimp) (Artenia sanfranciscoma)<br>Peraecus japoniesu (Kuruma pravni) (Marragenenesi japoniesu)<br>Eriocheir sinensis (Chinese mitten crab)<br>Calinetests sapidas (Blue crab)<br>Limulus polyphemus (Atlantic horseshoe erab)<br>Caricomes meensei (Common shore crab) (Gree erab)<br>Pealograpus mermoratus (Marbiel orck crab) (Cancer majster)<br>Penaesus monodor (Giant tiger pravn)<br>Metacerchus magister (Dungeness crab) (Cancer majster)<br>Metagenesse erabi (Greasyback shrimp) (Penaesus vonnodori,<br>Lia (Giant tiger pravn)<br>Metagenesse erabi (Greasyback shrimp) (Penaesus vonnomet)<br>Lia crassips (Fiddler crab)   | 284<br>284<br>284<br>284<br>284<br>284<br>355<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357  |
| А50648<br>A7L5V3<br>VSNBV4<br>M116607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51545<br>Q9N1449<br>P51541<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>C7E5T4<br>E7CGC2<br>A0A097KV73<br>D7F1J5<br>B1PV29<br>A0A097KVS3<br>Q004B5<br>A0A088FIL9  | unreviewed<br>unreviewed<br>versiewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed   | Limilae polyhemus (Allanic horseshoe enb)<br>Portums tribuceulands (Swimmigne enb) (Argenus tribuerulatus)<br>Macrobrachian resubregii (Giant fieth water pravn)<br>Pornamus pelagicae (Idles weinnet enb)<br>Ponneropenaeus mergaiensis (Banana pravn) (Penaeus mergaiensis)<br>Organism<br>Artenia franciscana (Brine shrimp) (Artennia sanfranciscana)<br>Penaeus iaponicus (Kunum pravn) (Marsupenaeus japonicus)<br>Eriocheir sinursis (Chiness mitten enb)<br>Calimetes sanjaha (Blue enb)<br>Limulus polyhemus (Atlantic horseshoe enb)<br>Carcinus meanas (Common shore enb) (Green enb)<br>Denaeus monodon (Giant tiger pravn)<br>Penaeus monodon (Giant tiger pravn)<br>Metacaercinus meafare (Daneses enb) (Cancer magister)<br>Caragen erangon (Brown shrimp)<br>Metaquenaeus envis (Greatyback shrimp) (Penaeus envis)<br>Metaquenaeus envis (Greatyback shrimp) (Penaeus envis)<br>Lica erasis (Fiddler enab)  | 284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357  |
| A5D648<br>A7L5V3<br>VSNBV4<br>M111607<br>D3XN80<br>P31545<br>Q9X148<br>Q9X148<br>Q9X148<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>D51541<br>Q9QYX1<br>C7E374<br>C7E374<br>D7F105<br>B1PV29<br>A0A097KV33<br>Q804B5  | unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyphemus (Atlantic horeschoe erab)<br>Portunas tribuceulands (Swimming erab) (Oxpannis tribuceulatus)<br>Macrobrachna rosenbergii (Giant fichs water pravn)<br>Pornorponaeta mergaietusi (Banasa pravn) (Peraecas mergaietusi)<br>Organism<br>Organism<br>Artenia franciscoma (Brine shrimp) (Artenia sanfranciscoma)<br>Peraecus japoniesu (Kuruma pravni) (Marragenenesi japoniesu)<br>Eriocheir sinensis (Chinese mitten crab)<br>Calinetests sapidas (Blue crab)<br>Limulus polyphemus (Atlantic horseshoe erab)<br>Caricomes meensei (Common shore crab) (Gree erab)<br>Pealograpus mermoratus (Marbiel orck crab) (Cancer majster)<br>Penaesus monodor (Giant tiger pravn)<br>Metacerchus magister (Dungeness crab) (Cancer majster)<br>Metagenesse erabi (Greasyback shrimp) (Penaesus vonnodori,<br>Lia (Giant tiger pravn)<br>Metagenesse erabi (Greasyback shrimp) (Penaesus vonnomet)<br>Lia crassips (Fiddler crab)   | 284<br>284<br>284<br>284<br>284<br>284<br>355<br>355<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>357  |
| А50648<br>A7L5V3<br>VSNBV4<br>M116607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51545<br>Q9N1449<br>P51541<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>Q9U5)4<br>C7E5T4<br>E7CGC2<br>A0A097KV73<br>D7F1J5<br>B1PV29<br>A0A097KVS3<br>Q004B5<br>A0A088FIL9  | unreviewed<br>unreviewed<br>unreviewed<br>versiewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed   | Limita polyhems (Alartic horeschoe erab)<br>Portums tribucadura (Swimmier erab)<br>Macrobrachian scale erab (Department indervalutars)<br>Macrobrachian resubergii (Giant fieth vater pravn)<br>Portums ribulgene (Blue swimmer erab)<br>Calibactica (Blue shima) (Artenia sanfranciscana)<br>Penaeus japonicus (Kuruma pravn) (Marsupenaeus japonicus)<br>Ericheir sinensi (Chinese milten erab)<br>Calibactes sapidas (Blue erab)<br>Limula polyhems (Alartic horeschoe erab)<br>Calibactes sapidas (Blue erab)<br>Limula polyhems (Alartic horeschoe erab)<br>Carcino soneras (Common shore erab) (Green erab)<br>Penaeus monodor (Giant tiger pravn)<br>Penaeus monodor (Giant tiger pravn)<br>Metaqueneus erab (Chaere magner)<br>Metaqueneus erab (Chaere magner)<br>Metaqueneus erab (Chaere magner)<br>Metaqueneus erab (Chaere hagt)<br>Metaqueneus erab (Chaere)<br>Lizagon erangen (Brown shrimp)<br>Metaqueneus erab (Greasyback shrimp) (Penaeus omannet)<br>Lizagoneus unsmet (Matelie princip) (Penaeus omannet)<br>Lizagoneus unsmet) (Havaitan red shrimp)  | 284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>284  |
| А50648<br>A7L5V3<br>VSNBV4<br>M11607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51541<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>C7E3T4<br>E7CGC2<br>A0A097KV37<br>D7FL15<br>B1PVZ9<br>A0A097KV83<br>Q004B5<br>A0A08FL9<br>Q4KY22  | unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyphemus (Atlantic horeschoe erab)<br>Portunus tribuceulanta (Swimming erab) (Neptanus tribucerulanta)<br>Macrobrachna moschogica (Giant (frei hvater pravn)<br>Peneroponaesa unergelentis (Banasa pravn) (Foreacas mergelentis)<br>Macrobrachna polegicae (Blue svinnar erab)<br>Ponaesa ioponicas (Blue svinnar erab)<br>Artenia franciscoma (Brine shtring) (Artenno enessi joponicas)<br>Eriocheir sinensis (Chinese milten erab)<br>Callineteris sapidas (Blue erab)<br>Limulas polyphemus (Atlantic horeschoe erab)<br>Carines meanza (Common shore erab) (Green erab)<br>Penaesa mondor (Giant (iger pravn)<br>Matacaercinus magister (Dangeness crab) (Cancer magnetister)<br>Carines meanza (Common shore erab) (Cancer magnetister)<br>Denaesa mondor (Giant (iger pravn)<br>Matacaercinus megister (Dangeness crab) (Cancer magnetister)<br>Caringon erangon (Brown shtring)<br>Matacaercinus megister (Dangeness crab) (Cancer magnetister)<br>Litogeneaes ensis (Gravaback shtring) (Penaesa vonnom)<br>Halocarintar nabra (Haveilat erab)<br>Litogeneaesa chinensi (Litogeneae chinensi)<br>Penaesa mondor (Dan tar grave)  | 284<br>284<br>284<br>285<br>285<br>355<br>355<br>357<br>357<br>357<br>357<br>356<br>210<br>356<br>210<br>356<br>356<br>356<br>356<br>356<br>356   |
| А50648<br>A7L5V3<br>VSNBV4<br>MIII607<br>D3XN80<br>P31545<br>Q9N148<br>Q9N148<br>Q9N148<br>Q9N148<br>Q9N149<br>P31541<br>Q0V34<br>Q9GYX1<br>C7E374<br>E7CGC2<br>A0A097KV71<br>D7F15<br>B1PV29<br>A0A097KV33<br>Q004B5<br>A0A085FL9<br>Q4KY22<br>G3D692<br>A0A097KV71   | unreviewed<br>unreviewed<br>unreviewed<br>verviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed   | Limina polyphemus (Allantic horeschoe erab)<br>Portunas tribuceulands (Swimming erab)<br>Macrobrachina rosenbergii (Giant fieth water pravn)<br>Pornoryphenesian pologicae (Bue swimmer erab)<br>Macrobrachina rosenbergii (Giant fieth water pravn)<br>Pomeruponnese mergeleistii (Banana pravn) (Poeseas mergeleinti)<br>Organism<br>Artenia franciscena (Brine shrimp) (Artenepaenese japonicus)<br>Peraesei gaponicus (Kurum peravn) (Marsupeneses japonicus)<br>Deriocheir sinensis (Chinese mitten erab)<br>Callineters sapidas (Blue erab)<br>Limulus polyphemus (Atlantic horseshoe erab)<br>Carcinos monosto (Giant iger pravn)<br>Penaesus monodon (Giant iger pravn)<br>Penaesus monodon (Giant iger pravn)<br>Metaponesus emis (Drangeness erab) (Cancer majster)<br>Carcinos megister (Dungeness erab) (Cancer majster)<br>Lear crassipse (Fiddler erab)<br>Litopenaesu svanodon (Giant iger pravn)<br>Metaponesus ensing (Greasyback shrimp) (Penaesu ensing)<br>Litopenaesu svanoging (Dialedi erab)<br>Litopenaesu svanomei (Whieleg shrinp) (Penaesu ensing)<br>Halocaridiar nihro (Havaiian red shrimp)<br>Formano prinkervaluta (Swimming erab) (Staputer triboreculatus)<br>Portunas tribuceulatus (Swimming erab) (Staputer introbreculatus)  | 284<br>284<br>284<br>284<br>355<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356  |
| А50648<br>A7L5V3<br>VSNBV4<br>MIII607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9NI449<br>P51545<br>Q9NI449<br>P51541<br>Q9U514<br>Q9U514<br>Q9U514<br>Q9U514<br>C7E5T4<br>E7CGC2<br>A0A097KV17<br>D7F1J5<br>BIPV29<br>A0A097KVS3<br>Q00H35<br>A0A088FL9<br>Q4KY22<br>G3D692<br>A0A097KVD1<br>A0A097KVS5   | unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyhems (Alartic horeschoe erab)<br>Portums triiboreadnas (Swimming erab) (Orpanus triiboreadnas)<br>Macrobrachian sosabergii (Giant fiels water pravn)<br>Portums pologicae (Bites swimmer erab)<br>Pomeruponaeae mergaiensis (Banana pravn) (Ponaeas mergaiensis)<br>Calinectos and Brite shrinp) (Artenia sonfranciscono)<br>Penaeas igonoicus (Kuruma pravn) (Marsuponaeas japonicus)<br>Ericcheir sinossis (Chinese milten erab)<br>Calinectos sapidos (Blue erab)<br>Limula polyhems (Alartic horeschoe erab)<br>Cacrims moranos (Common solvee erab) (Green erab)<br>Pondograpus marnoratus (Mattelo drock erab) (Cancer mamoratus)<br>Pondograpus marnoratus (Mattelo drock erab) (Cancer mamoratus)<br>Matageneaes eras (Cancups (Erab)<br>Matageneaes eras (Cancups (Erab))<br>Matageneaes eras (Cancups (Erab)<br>Lingun eras eras (Cancups (Erab))<br>Lingun eras usonnoid (Mittel ge travn)<br>Lingun eras usonnoid (Mittel ge travn)<br>Halocaridina rabra (Hasviaian red shrimp)<br>Portumas triuberealatus (Swimming erab) (Nepanas chinesus)<br>Atergapis integerrinus (Edel ge carb) (Cancer martinberealatus)<br>Portumas triuberealatus (Swimming erab) (Nepanas chinesus)   | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>357<br>210<br>356<br>356<br>356<br>356<br>357<br>210<br>356        |
| А50648<br>A7L5V3<br>VSNBV4<br>MIII607<br>D3XN80<br>P31545<br>Q9N148<br>Q9N148<br>Q9N148<br>Q9N148<br>Q9N149<br>P31541<br>Q0V34<br>Q9GYX1<br>C7E374<br>E7CGC2<br>A0A097KV71<br>D7F15<br>B1PV29<br>A0A097KV33<br>Q004B5<br>A0A085FL9<br>Q4KY22<br>G3D692<br>A0A097KV71   | unreviewed<br>unreviewed<br>unreviewed<br>verviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed   | Limina polyphemus (Allantic horeschoe erab)<br>Portunas tribuceulands (Swimming erab)<br>Macrobrachina rosenbergii (Giant fieth water pravn)<br>Pornoryphenesian pologicae (Bue swimmer erab)<br>Macrobrachina rosenbergii (Giant fieth water pravn)<br>Pomeruponnese mergeleistii (Banana pravn) (Poeseas mergeleinti)<br>Organism<br>Artenia franciscena (Brine shrimp) (Artenepaenese japonicus)<br>Peraesei gaponicus (Kurum peravn) (Marsupeneses japonicus)<br>Deriocheir sinensis (Chinese mitten erab)<br>Callineters sapidas (Blue erab)<br>Limulus polyphemus (Atlantic horseshoe erab)<br>Carcinos monosto (Giant iger pravn)<br>Penaesus monodon (Giant iger pravn)<br>Penaesus monodon (Giant iger pravn)<br>Metaponesus emis (Drangeness erab) (Cancer majster)<br>Carcinos megister (Dungeness erab) (Cancer majster)<br>Lear crassipse (Fiddler erab)<br>Litopenaesu svanodon (Giant iger pravn)<br>Metaponesus ensing (Greasyback shrimp) (Penaesu ensing)<br>Litopenaesu svanoging (Dialedi erab)<br>Litopenaesu svanomei (Whieleg shrinp) (Penaesu ensing)<br>Halocaridiar nihro (Havaiian red shrimp)<br>Formano prinkervaluta (Swimming erab) (Staputer triboreculatus)<br>Portunas tribuceulatus (Swimming erab) (Staputer introbreculatus)  | 284<br>284<br>284<br>284<br>355<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356  |
| А50648<br>A7L5V3<br>VSNBV4<br>MIII607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9NI449<br>P51545<br>Q9NI449<br>P51541<br>Q9U514<br>Q9U514<br>Q9U514<br>Q9U514<br>C7E5T4<br>E7CGC2<br>A0A097KV17<br>D7F1J5<br>BIPV29<br>A0A097KVS3<br>Q00H35<br>A0A088FL9<br>Q4KY22<br>G3D692<br>A0A097KVD1<br>A0A097KVS5   | unreviewed<br>unreviewed<br>unreviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed  | Limita polyhems (Alartic horeschoe erab)<br>Portums triiboreadnas (Swimming erab) (Orpanus triiboreadnas)<br>Macrobrachian sosabergii (Giant fiels water pravn)<br>Portums pologicae (Bites swimmer erab)<br>Pomeruponaeae mergaiensis (Banana pravn) (Ponaeas mergaiensis)<br>Calinectos and Brite shrinp) (Artenia sonfranciscono)<br>Penaeas igonoicus (Kuruma pravn) (Marsuponaeas japonicus)<br>Ericcheir sinossis (Chinese milten erab)<br>Calinectos sapidos (Blue erab)<br>Limula polyhems (Alartic horeschoe erab)<br>Cacrims moranos (Common solvee erab) (Green erab)<br>Pondograpus marnoratus (Mattelo drock erab) (Cancer mamoratus)<br>Pondograpus marnoratus (Mattelo drock erab) (Cancer mamoratus)<br>Matageneaes eras (Cancups (Erab)<br>Matageneaes eras (Cancups (Erab))<br>Matageneaes eras (Cancups (Erab)<br>Lingun eras eras (Cancups (Erab))<br>Lingun eras usonnoid (Mittel ge travn)<br>Lingun eras usonnoid (Mittel ge travn)<br>Halocaridina rabra (Hasviaian red shrimp)<br>Portumas triuberealatus (Swimming erab) (Nepanas chinesus)<br>Atergapis integerrinus (Edel ge carb) (Cancer martinberealatus)<br>Portumas triuberealatus (Swimming erab) (Nepanas chinesus)   | 284<br>284<br>284<br>284<br>356<br>355<br>357<br>357<br>357<br>357<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>357<br>210<br>356<br>356<br>356<br>356<br>357<br>210<br>356        |
| А50648<br>A7L5V3<br>VSNBV4<br>MII607<br>D3XNS0<br>Entry (arginine kinase<br>Q95V58<br>P51545<br>Q9N1449<br>P51541<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>Q9U344<br>D7E115<br>B1PV29<br>A0A097KV33<br>Q004B5<br>A0A08FKU5<br>A0A08FKU5<br>H0UK50  | unreviewed<br>unreviewed<br>unreviewed<br>versiewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>reviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed<br>unreviewed     | Limina polyphemus (Altanic horeschoc erab)<br>Portums tribuceulanta (Swimming erab) (Oxpannar kinderculants)<br>Macrobrachian resubergii (Giant fieth water pravn)<br>Pornama pologicae (Biles winner erab)<br>Pomerupenaeus mergelensis (Banana pravn) (Ponaeus mergelensis)<br>Demos (Brine Shrinp) (Atrendis surfranciscono)<br>Artenia franciscona (Brine Shrinp) (Atrendis surfranciscono)<br>Deficientes supidas (Biles erab)<br>Artenia franciscona (Brine Shrinp) (Atrendis surfranciscono)<br>Deficientes supidas (Biles erab)<br>Artenia franciscona (Brine Shrinp) (Atrendis surfranciscono)<br>Deficientes supidas (Biles erab)<br>Callinectes supidas (Biles erab)<br>Callinectes supidas (Biles erab)<br>Carcinus memas (Common shore erab) (Green erab)<br>Penaeus monodor (Giant tiger pravn)<br>Penaeus monodor (Giant tiger pravn)<br>Penaeus monodor (Giant tiger pravn)<br>Penaeus monodor (Giant tiger pravn)<br>Cargon erangon (Brown shrinp)<br>Matopenaeus enusi (Greaxyback shrinp) (Penaeus enusi)<br>Lie caressipter (Fiddle erab)<br>Libenonaeus enusis (Fieldby pravn) (Penaeus enusint)<br>Penneropenaeus chinensis (Fieldby pravn) (Penaeus chinensis)<br>Penneropenaeus chinensis (Fieldby pravn) (Penaeus chinensis)<br>Penneropenaeus chinensis (Fieldby pravn) (Penaeus chinensis)<br>Pennaeus triubereulants (Winthing erab) (Magnum a triubereulants)<br>Portnaus pelagicar (Belag genab) (Cancer integerinus)<br>Portnaus pelagicar (Belag genab) (Cancer integerinus)   | 284<br>284<br>284<br>284<br>356<br>357<br>357<br>357<br>357<br>357<br>357<br>357<br>356<br>356<br>210<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>210<br>210<br>210<br>210<br>210 |
| А50648<br>A7L5V3<br>VSNBV4<br>MIII607<br>D3XN80<br>P31545<br>Q9XV8<br>Q9XV8<br>Q9XV8<br>Q9X148<br>Q9X148<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X144<br>Q9X147<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374<br>C7E374 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| Limita polyphemus (Atlantic horeschoe erab)<br>Portunus tribuevalutas (Swimmig erab) (Neptanus tribuevalutas)<br>Macrobrachna rosenbergii (Giant firsh water pravn)<br>Penenroponaesa unergeiettii (Banausa pravn) (Peneaus merguientii)<br>Organism<br>Organism<br>Artenia franciscoma (Kinus Burnpi) (Artennaesas japonicus)<br>Eriocheir sinensis (Chinese mitten erab)<br>Limitas polyphemus (Atlantic horseshoe erab)<br>Limitas polyphemus (Atlantic horseshoe erab)<br>Caclineetes sapidus (Bue erab)<br>Limitas polyphemus (Atlantic horseshoe erab)<br>Caclineetes sapidus (Bue erab)<br>Limitas polyphemus (Atlantic horseshoe erab)<br>Caclineetes sapidus (Bue erab)<br>Limitas polyphemus (Atlantic horseshoe erab)<br>Caclines maenza (Common shore erab) (Green erab)<br>Peneaus monodon (Giant tiger pravn)<br>Matacarcinus magister (Dangeness erab) (Cancer marjoster)<br>Carangen erangon (Brown shrimp)<br>Matageneaus ensis (Greavyback shrimp) (Penaeus vonname)<br>Litopenaeus vonnamei (Whiteleg shrimp) (Penaeus vonname)<br>Halocaridium erabo (Hawiain et shrimp)<br>Penneropenaeus chinensis (Flestby pravn) (Kenaeus triuberculatus)<br>Ategatis integerirmus (Red etg erab) (Cancer integerirmus)<br>Ategatis integerirmus (Red etg erab) (Cancer integerirmus)<br>Asyla paramamesain (Mud erab) | 284<br>284<br>284<br>284<br>284<br>284<br>284<br>284<br>285<br>285<br>210<br>210<br>210<br>210<br>256<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>356<br>3                 |



The specificity of targeted signature peptides was confirmed by the Basic Local Alignment Search Tool (BLAST) of Uniprot (http://www.uniprot.org/blast). It is critical that signature

peptides are unique to the target protein and detectable by the MS systems of choice.<sup>10</sup> The more information is available, the higher the certainty that the peptide represents the allergen and species of interest. Unfortunately, because of the sequence homology of allergens in different species, it is not always possible that signature peptides are species specific. The results of cross-reaction with TM and AK from other organisms by BLAST were listed in Table 2.

The selected signature peptides do not need to be the most intense signals found in MS spectra, but they do need to be sufficiently intense to allow clear separation from other peptides or MS background (Fig. 3). The heat stability of the selected signature peptides were tested at 100 °C for 0.5 h. There is no significantly change (P < 0.05, data not shown here) to the intensity of each peptide.

#### Digestion by trypsin

When identifying and detecting allergen proteins using MS systems, sample preparation is a critical step. Allergen proteins are usually required to be extracted from food matrice and digested with enzymes generating peptides. For comparison, molecular cut-off filter was used after the protein extraction with Tris-HCl solution as described by previous reports.19-21 However, there is no obvious difference in the numbers of obtained peptides. For clean-up of obtained peptides, SPE was adopted. A systematic investigation of orthogonal SPE clean-up of digested samples was performed by Yuan et al.22 In this study, Oasis® HLB SPE column was used for purification.

Various enzymes are available with specific cleavage sites. Trypsin was adopted in our experiment. It is the most commonly used enzyme due to the well-known cleavage sites between the amino acid arginine (R) and lysine (K). Carrera et al.19 has approved that more peptides could be identified when fish species were digested with trypsin compared with Glu-C.

#### Method validation

For validation of the detection ability, the home-made fish balls containing 1.5% shrimp meat were selected as the QC sample.

| Table 2 Resul      | ts of surrogate peptide | es by BLAST in UniProt   |
|--------------------|-------------------------|--|
| Protein            | Surrogate peptide       | Cross-reaction from<br>other organism by BLAST   |
| Tropomyosin        | ALSNAEGEVAALNR          | Sinonovacula constricta,<br>Tyrophagus putrescentiae,<br>Portunus pelagicus, Paralithodes<br>camtschaticus, et al.   |
| Arginine<br>kinase | VSSTLSSLEGELK           | Drosophila mojavensis, Portunus<br>trituberculatus, Macrophthalmus<br>japonicas, Portunus<br>trituberculatus, Larinus sp.<br>BHJ-2011, Calcinus<br>laevimanus, Aegla neuquensis,<br>Gomeza bicornis, Dotilla<br>myctiroides, Eplumula<br>phalangium, Hylaeus elegans,<br>Orithyia sinica, et al. |

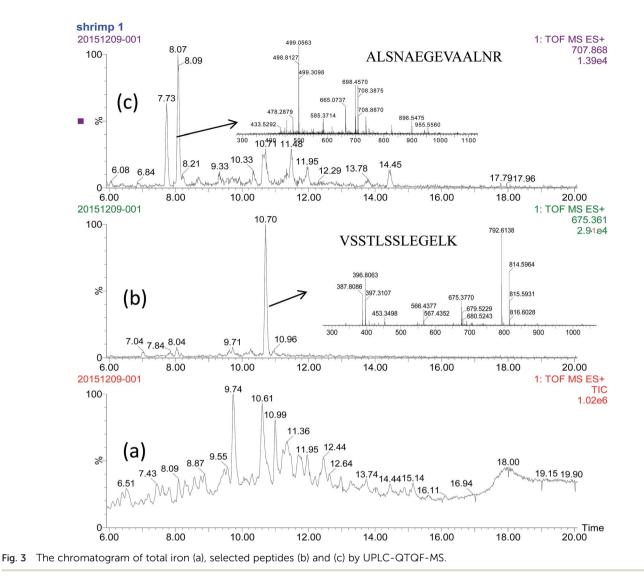


Table 3 The precision, accuracy and linearity of surrogate peptide for quantification in UPLC-QToF-MS

|                 | Surrogate peptide | Precursor $\binom{2^+}{product}$ ion $\binom{1^+}{product}$ | Correlation coefficient<br>(r) | Intraday $(n = 3)$ |      | Interday $(n = 3)$ |     |
|-----------------|-------------------|---|--------------------------------|--------------------|------|--------------------|-----|
| Protein         |                   |   |                                | R                  | RSD  | R                  | RSD |
| Tropomyosin     | ALSNAEGEVAALNR    | 707.868/829.452   | 0.9912                         | 86                 | 9.5  | 90                 | 7.5 |
| Arginine kinase | VSSTLSSLEGELK     | 675.361/862.451   | 0.9909                         | 88                 | 11.3 | 92                 | 8.1 |

As shown in Table 3, the RSDs of inter- and intra-day were all less than 15%, and the recoveries were more than 80% (Table 3). Usually, the level owning mass response of a signal-to-noise ratio (S/N) of 3 is considered as limit of detection. In this study, the detection limit, expressed of shrimp meat per kilogram of food, was 8 g kg<sup>-1</sup> (usage of TM) or 5 g kg<sup>-1</sup> (AK).

# Conclusion

The shrimp allergen proteins (tropomyosin and arginine kinase) in complex foodstuffs can be fast detected by in-solution

digestion and UPLC-QTQF-MS. The surrogate peptides, ALS-NAEGEVAALNR for TM and VSSTLSSLEGELK for AK were screened. The developed method is suitable to screen potential addition of shrimp muscle in foodstuffs by detection of allergen proteins.

Furthermore, it should be noted that the present method was only used for screening shrimp muscle *via* allergenic proteins in complex foodstuffs. Although different crustacean have species-specific peptides for TM, AK or other proteins,<sup>23</sup> selected surrogate peptides of ALSNAEGEVAALNR and VSSTLSSLEGELK have cross-reaction with other organisms,

especially for some crabs (for example *Portunus trituberculatus* and *Calcinus laevimanus*) (Table 2). Our further work will focus on the screening of a generic surrogate peptide in TM or AK for quantifying most of crustacean foods.

# Author contributions statement

X.-D. P, Q. C and B.-F. H conceived the experiment(s), X.-D. P, conducted the experiment(s), X.-D. P and B.-F. H analyzed the results. All authors reviewed the manuscript.

# Conflicts of interest

The authors declare no competing financial interests.

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