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# Specific Detection of Potentially Allergenic Kiwifruit in Foods Using Polymerase Chain Reaction

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Kiwifruit (*Actinidia deliciosa* and *Actinidia chinensis*) is allergenic to sensitive patients, and, under Japanese regulations, it is one of the food items that are recommended to be declared on food labeling as much as possible. To develop PCR-based methods for the detection of trace amounts of kiwifruit in foods, two primer pairs targeting the ITS-1 region of the *Actinidia* spp. were designed using PCR simulation software. On the basis of the known distribution of a major kiwifruit allergen (actinidin) within the *Actinidia* spp., as well as of reports on clinical and immunological cross-reactivities, one of the primer pairs was designed to detect all *Actinidia* spp. and the other to detect commercially grown *Actinidia* spp. (i.e., kiwifruit, *Actinidia arguta*, and their interspecific hybrids) except for *Actinidia polygama*. The specificity of the developed methods using the designed primer pairs was verified by performing PCR experiments on 8 *Actinidia* spp. and 26 other plants including fruits. The methods were considered to be specific enough to yield target-size products only from the target *Actinidia* spp. and to detect no target-size products from nontarget species. The methods were sensitive enough to detect 5−50 fg of *Actinidia* spp. DNA spiked in 50 ng of salmon testis DNA used as a carrier (1−10 ppm of kiwifruit DNA) and 1700 ppm (w/w) of fresh kiwifruit puree spiked in a commercial plain yogurt (corresponding to ca. 10 ppm of kiwifruit protein). These methods would be expected to be useful in the detection of hidden kiwifruit and its related species in processed foods.

**KEYWORDS: Food allergy; kiwifruit; *Actinidia* spp.; internal transcribed spacer; ITS; PCR**

## INTRODUCTION

Kiwifruit (*Actinidia deliciosa* cv. Hayward and *Actinidia chinensis* cv. Hort16A) is a major fruit that is cultivated extensively in New Zealand, Italy, Chile, France, Greece, and other subtropical areas including Japan. Kiwifruit allergy is one of the most important fruit allergies because of its serious symptoms and because many clinical cases have been reported worldwide (*1*−*3*). The general symptoms of the kiwifruit allergy are urticaria and oral allergy syndrome (OAS), the latter of which includes such symptoms as oral and pharyngeal itching, oral papules or blisters, lip irritation and swelling, labial edema, and glottis edema (*1*, *4*). Many clinical cases of kiwifruit allergy have been reported in Europe (*5*) and Japan (*6*, *7*), and the ingestion of a trace amount of kiwifruit induces the symptoms in sensitive patients (*8*).

In addition to kiwifruit, some other *Actinidia* spp. are also cultivated for food and distributed in the market (*9*). For example, a cultivar of *Actinidia arguta* (sarunashi), commercially known as baby kiwi, is grown and distributed in many countries. In addition, *Actinidia polygama* (matatabi) and interspecific hybrids of *Actinidia arguta* × *A. deliciosa* are also grown and consumed in Japan in the form of fresh fruit, juice, jam, and so on.

Some of the allergen molecules in kiwifruit have been reported (*10*). One of the major ones, actinidin (Act c 1), was also reported to be present in *A. arguta* (*11*−*13*). Although there have been few reports of the presence of kiwifruit allergen in *A. polygama*, the intake of *A. polygama* by patients with kiwifruit allergy might induce the allergy.

According to Japanese food labeling regulations, five food items (wheat, buckwheat, egg, milk, and peanut) must be declared on food labeling, and several detection methods for these items have been reported (*14*, *15*). In addition to these 5 items, a Japanese ordinance recommends that 20 food items, including kiwifruits, should be declared on food labeling as far as possible. In terms of these 20 food items, the detection

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methods remain to be developed. To assess the validity of food labeling and to ensure the safety of allergic patients, detection methods for these 20 items are indispensable.

Major techniques used in the detection of allergenic foods are ELISA and PCR. Both techniques have advantages and disadvantages. Whereas most of the ELISA methods target a specific allergenic protein and usually some cross-reactivity could occur, PCR targets a specific DNA sequence to detect the presence of the offending food. Therefore, PCR would be suitable for a final identification method of the presence of an allergenic ingredient for food labeling. In addition, it is generally thought that the damage done to DNA during food processing is relatively less compared to the damage done to proteins (*16*). In Japan, it is either mandatory or recommended to declare allergenic ingredients on food labeling when 10 ppm ($\mu$g/g or $\mu$g/mL) or more total protein of an allergenic food is present. As an allergenic food detection method, Japanese regulation specifies PCR for final identification of the presence of some allergenic ingredients after initial screening determination by ELISA. Both methods would be complementary to each other and should be necessary for an accurate allergenic ingredient testing.

The internal transcribed spacer (ITS) used for our PCR target is located between 18S (small subunit) and 26S (large subunit) nuclear ribosomal RNA genes, which include two spacers (ITS-1 and ITS-2) separated by the 5.8S rRNA gene. The ITS is known to be present in large copy numbers on genomic DNA and to be useful for congeneric or conspecific classifications (*17*−*19*). Use of these regions allows us to detect a target plant species with high sensitivity and specificity (*20*).

In the present study, we designed two primer pairs for PCR-based kiwifruit detection methods using ITS-1 as the target region; one was designed for detecting all of the *Actinidia* spp. and the other for detecting commercially grown *Actinidia* spp. but excluding *A. polygama*. The specificity, sensitivity, and analytical results from experiments using the developed methods to detect kiwifruit or sarunashi in several commercial products are reported.

## MATERIALS AND METHODS

**Samples Used in DNA Isolation.** Two kiwifruits (*A. deliciosa* cv. Hayward and *A. chinensis* cv. Hort16A), two fruits of tara vine [sarunashi in Japanese (*A. arguta* cv. Issai, an unknown cultivar of *A. arguta* marketed as baby kiwi)], one interspecific hybrid of kiwifruit named Sanuki gold, two interspecific hybrids of *A. arguta* × *A. deliciosa* named Kosui and Shinzan, and one fruit of silver vine [matatabi in Japanese (*A. polygama*)] were purchased from local markets and farms in Japan. Apples (*Malus domestica*), aloe plants (*Aloe arborescens*), apricots (*Prunus armeniaca*), avocados (*Persea americana*), bananas (*Musa acuminata*), blueberries (*Vaccinium* spp.), cherries (*Prunus avium*), figs (*Ficus carica*), grapes (*Vitis* spp.), persimmons (*Diospyros kaki*), mangos (*Mangifera indica*), melons (*Cucumis melo*), oranges (*Citrus sinensis*), papayas (*Carica papaya*), peaches (*Prunus percica*), pears (*Pyrus communis*), pineapples (*Ananas comosus*), prunes (*Prunus salicina*), raspberries (*Rubus idaeus*), satsuma oranges (*Citrus unshu*), strawberries (*Fragaria* × *ananassa*), Japanese apricots (*Prunus mume*), corn (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), and wheat (*Triticum aestivum*) were purchased at local supermarkets in Chiba and Tokyo, Japan. Some commercial products containing kiwifruit or sarunashi were also purchased, namely, a cereal with a dried fruit mix, a kiwifruit cookie, dried kiwifruits, gummy candies (assorted fruit flavors), a kiwifruit jam, a sarunashi jam, three kinds of juice or fruit drinks (100% kiwifruit juice, mixed fruits including kiwifruit, and 10% sarunashi juice), and two kinds of yogurt (one with pieces of mixed fruit and the other with pieces of kiwifruit only). Finally, some commercial products without kiwifruit or sarunashi in the list of ingredients, namely, a cereal with a dried fruit mix, a cookie with grapefruit jam, and fruit and vegetable drinks, were also purchased at local supermarkets in Chiba and Tokyo, Japan.

**Primer Design.** The DNA sequences of the ITS-1 region were used for the primer design. Twenty-eight sequences of the family Actinidiaceae (including 26 *Actinidia* spp.) and 29 sequences of plants used for food (including fruits) were obtained from GenBank. Because the sequences of banana (*Musa acuminata*), fig (*Ficus carica*), and persimmon (*Diospyros kaki*) were not found in GenBank, the sequences obtained from congeneric species of those fruits were used for the PCR simulations. When there was more than one ITS-1 sequence reported for an *Actinidia* species, the sequence most homologous to the *A. deliciosa* ITS-1 sequence was selected as the representative of that species. In addition, ITS-1 sequences of *A. deliciosa* cv. Hayward, *A. chinensis* cv. Hort16A, *A. arguta* cv. Issai, an unknown cultivar of *A. arguta* (baby kiwi), and *A. polygama* (matatabi) purchased for this study were determined by a direct sequencing method. Two sets of primer pairs (the F151 and R182 primer pair and the F123 and R178 primer pair) were designed on the basis of the highly homologous sequences among the target *Actinidia* spp. in the ITS-1 region. PCR simulations were performed with Amplify 1.0 software (Bill Engels, University of Wisconsin) to predict whether PCR products of the target size would be obtained from the DNA sequences of ITS-1 reported for 33 *Actinidia* spp., 2 species of the family Actinidiaceae other than *Actinidia* spp., and 29 other plants listed in **Table 1**. The primer pair of CP03-F (5′-CGG ACG AGA ATA AAG ATA GAG T-3′) and CP03-R (5′-TTT TGG GGA TAG AGG GAC TTG A-3′), designed to amplify a partial region of plant chloroplast DNA (*21*), was used to validate the quality of extracted DNA as templates. The primers were synthesized and purified with an oligonucleotide purification cartridge by Operon Biotechnologies, Inc.

**DNA Extraction from Plants.** Plant materials (the flesh of fruits, seeds, or leaves depending on the samples) were homogenized using an MM300 mixer mill (Retsch, Haan, Germany). DNA was extracted from 2 g of homogenized sample with 20 mL of buffer G2 (Qiagen, Hilden, Germany) and purified using Genomic-tip 20/G (Qiagen) according to the manufacturer's instructions. The DNA concentrations were determined by measuring the UV absorption at 260 nm. All DNA solutions were diluted to 20 ng/$\mu$L with TE (pH 8.0) and used for PCR templates. For the sensitivity studies, DNA solutions of *Actinidia* spp. were further diluted with 5 ng/$\mu$L salmon testis DNA (Sigma Chemical Co., St. Louis, MO) solution. In addition, *A. deliciosa* cv. Hayward DNA was diluted with 20 ng/$\mu$L of salmon testis DNA to obtain 0.1−10 ppm (wt/wt) of kiwifruit DNA in 50 ng of salmon testis DNA (the carrier DNA) and used for PCR templates.

**DNA Extraction from Commercial Products and Kiwifruit-Spiked Sample.** An entire pack of each commercial product was homogenized using a mixer mill (IMF-300; Iwatani International Corp., Tokyo, Japan). Then, DNA was isolated from the homogeneous mixture according to the same method using Genomic-tip 20/G (Qiagen) as described above (×1 DNA extraction scale). Because the DNA samples extracted from kiwifruit jam, kiwifruit juice, and gummy candies did not consistently yield the expected PCR products, the amount of the sample and buffer G2 was increased 10 times, and the extracted DNA was purified using Genomic-tip 100/G (×10 DNA extraction scale). To obtain amplifiable DNA from dried kiwifruit, it was first necessary to wash off the surface coating with distilled water. For the sensitivity studies, DNA was also isolated from a commercial plain yogurt spiked with 1700 ppm (w/w) of fresh kiwifruit (Hayward) puree (0.6% protein content measured with a 2-D Quant kit; GE Healthcare U.K. Ltd., Little Chalfont, U.K.). The DNA concentrations were determined by measuring the UV absorption at 260 nm and adjusted to 20 ng/$\mu$L with TE (pH 8.0) for PCR. In most cases, however, the DNA samples extracted from the commercial products were less than 20 ng/$\mu$L and were directly subjected to PCR without dilution.

**PCR.** PCR was carried out in a 25 $\mu$L reaction volume containing 0.2 mM of each dNTP, 1× buffer (PCR buffer II), 1.5 mM $MgCl_2$, 0.625 unit of AmpliTaq Gold (Applied Biosystems, Foster City, CA), 0.5 $\mu$M of each primer pair (the F151 and R182 primer pair or the F123 and R178 primer pair), and 5 fg−50 ng of template DNA. The amplifications were performed in a GeneAmp PCR system 9600

**Table 1.** Specificity Prediction of Primer Pairs (**A**, F151 and R 182; **B**, F123 and R178) with PCR Simulation Software

| species (common name) | GenBank Accession no. | weight no.[a] (amplicon size)[b] (**A**) F151 and R182 primer pair | weight no.[a] (amplicon size)[b] (**B**) F123 and R178 primer pair |
|---|---|---|---|
| family Actinidiaceae | | | |
| genus *Actinidia* | | | |
| *A. deliciosa* (Hayward) | AB253775 | 6*[c] | 6* |
| *A. chinensis* (Hort16A) | AB253776 | 5* | 6* |
| *A. deliciosa* | AF323830 | 6* | 6* |
| *A. arguta* (Issai) | AB253777 | 5* | 5* |
| *A. arguta* (baby kiwi) | AB253778 | 5* | 6* |
| *A. arguta* | AY216736 | 5* | 6* |
| *A. arguta* | AF323836 | 5* | 6* |
| *A. arguta* | AF323835 | 5* | 6* |
| *A. polygama* (matatabi) | AB253779 | 5* | – |
| *A. polygama* | AF323796 | 5* | – |
| *A. callosa* | AF323829 | 6* | 6 |
| *A. chrysantha* | AF323797 | 5* | 6 |
| *A. cylindrica* | AF323807 | 5* | 6 |
| *A. eriantha* | AF323801 | 5* | 6 |
| *A. fulvicoma* | AF323799 | 5* | 6 |
| *A. glaucophylla* | AF323798 | 5* | 6 |
| *A. hemsleyana* | AF323802 | 4* | – |
| *A. henanesis* | AF323841 | 6* | 6 |
| *A. indochinensis* | AF323810 | 5* | 6 |
| *A. kolomikta* | AF323837 | 5*(75 bp) | – |
| *A. latifolia* | AF323825 | 5* | 6 |
| *A. macrosperma* | AF323834 | 5* | 6 |
| *A. melanandra* | AF443211 | 5* | 6 |
| *A. melliana* | AF323821 | 5* | 5 |
| *A. persicina* | AF323814 | 5* | – |
| *A. rufa* | AF323838 | 5* | 6 (93 bp) |
| *A. rufa* | AF323839 | 5* | 5 (93 bp) |
| *A. sabiifolia* | AF323813 | 5* | 6 |
| *A. styracifolia* | AF323822 | 5* | 6 |
| *A. valvata* | AF323842 | 5* | 6 |
| *A. zhejiangensis* | AF323819 | 5* | – |
| other genera | | | |
| *Clematoclethra lasioclada* | AF323805 | – | – |
| *Saurauia zahlbruckneri* | AF396452 | – | – |
| plants used for food (containing major fruits) | | | |
| *Aloe vera* (aloe) | AF234345 | – | – |
| *Carica papaya* (papaya) | AY461547 | – | – |
| *Cucumis melo* (melon) | CME488233 | 2 (49 bp) | – |
| *Diospyros whyteana* (relative of persimmon) | AF396234 | – | – |
| *Ficus tonduzii* (relative of fig) | AY730140 | – | – |
| *Fragaria* × *ananassa* (strawberry) | AF163494 | – | – |
| *Malus domestica* (apple) | MDU16195 | – | – |
| *Mangifera indica* (mango) | AB071674 | – | – |
| *Musa beccarii* (relative of banana) | AF434900 | – | – |
| *Persea americana* (avocado) | AF272322 | 2 (39 bp) | – |
| *Prunus armeniaca* (apricot) | AF318756 | – | – |
| *Prunus avium* (cherry) | AF318737 | – | – |
| *Prunus domestica* (plum) | AF318713 | – | – |
| *Prunus mume* (Japanese apricot) | AF318728 | – | – |
| *Prunus persica* (peach) | AF318741 | – | – |
| *Pyrus calleryana* (pear) | PCU16202 | – | – |
| *Pyrus pyrifolia* (pear) | AF287247 | – | – |
| *Pyrus salicifolia* (relative of pear) | AF186532 | – | – |
| *Rubus idaeus* (raspberry) | AF055757 | – | – |
| *Vaccinium corymbosum* (blueberry) | AF419778 | – | – |
| *Vaccinium uliginosum* (blueberry) | DQ217769 | – | – |
| *Vitis rotundifolia* (grape) | AY037922 | – | – |
| *Vitis vinifera* (grape) | AF365988 | – | – |
| *Arachis hypogaea* (peanut) | AF156675 | – | – |
| *Fagopyrum esculentum* (soba) | AB000330 | – | – |
| *Glycine max* (soybean) | AF144654 | – | – |
| *Oryza sativa* (rice) | AF169230 | – | – |
| *Triticum aestivum* (wheat) | AM040486 | – | – |
| *Zea mays* (corn) | U46648 | – | – |

[a] An approximate guide to the quality of the matches and the strength of the amplifications. The larger the weight number (1–6), the higher the probability of amplification. A dash (–) indicates no amplicon was predicted. [b] The predicted size of the amplicon, which is different from the target size. The amplicon sizes reported here are 2 bp shorter than those predicted by Amplify 1.0, which takes into account the terminal transferase activity of DNA polymerase (F151 and R182, 74 bp; F123 and R178, 92 bp). [c] An asterisk (*) indicates the target *Actinidia* spp. in each PCR primer pair.

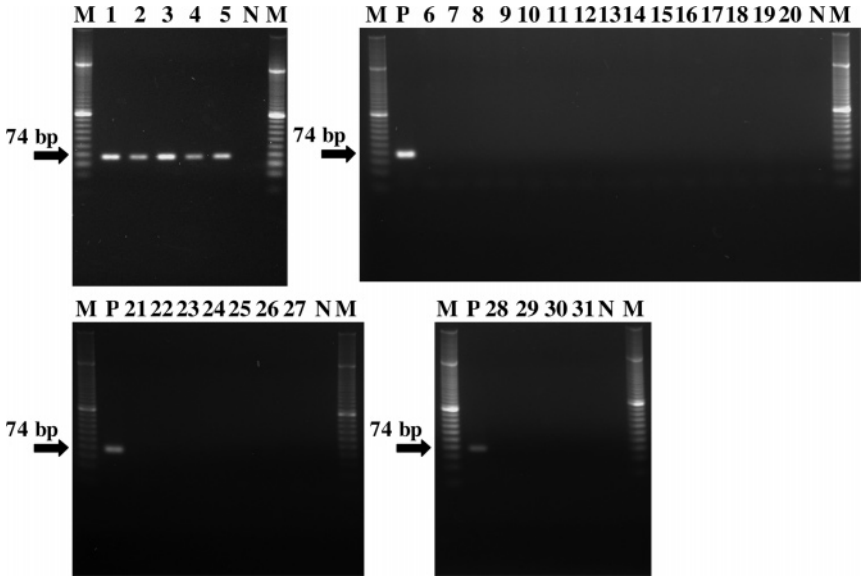


**Figure 1.** Specificity of the F151 and R182 primer pair for all *Actinidia* spp. The arrowhead indicates the expected PCR product. M, DNA marker (20 bp ladder, Takara Bio Inc.); P, amplification of 500 pg of Hayward genomic DNA as a positive control; N, negative control (no template). (Lanes 1–31) Amplification of 50 ng of Hayward (1), Hort16A (2), Issai (3), baby kiwi (4), matatabi (5), aloe (6), pineapple (7), papaya (8), orange (9), satsuma orange (10), melon (11), persimmon (12), fig (13), strawberry (14), apple (15), mango (16), banana (17), avocado (18), apricot (19), cherry (20), Japanese apricot (21), peach (22), prune (23), pear (24), raspberry (25), blueberry (26), grape (27), wheat (28), rice (29), soybean (30), and corn (31) genomic DNA.

(Applied Biosystems) as follows: preincubation at 95 °C for 10 min; 50 cycles consisting of denaturation at 95 °C for 0.5 min, annealing at 60 °C for 0.5 min, and extension at 72 °C for 0.5 min; and a final extension at 72 °C for 7 min. When the F123 and R178 primer pair was used, the number of PCR cycles was reduced to 40. The PCR products (5 $\mu$L) were electrophoresed on a 3% agarose gel containing ethidium bromide and analyzed with a ChemiDoc XRS illuminator (Bio-Rad Laboratories, Inc., Hercules, CA). Sensitivity studies for each primer pair were performed in eight replicate runs. All of the DNA samples used for the specificity and sensitivity studies gave the expected PCR products with the CP03-F and CP03-R primer pair used for the quality validation of the DNA (*21*).

## RESULTS

**Primer Design for Detection of Kiwifruit.** Two sets of primer pairs were designed. One primer pair was designed to detect all of the *Actinidia* spp. including kiwifruit, *A. arguta*, and *A. polygama*. The other was designed to detect kiwifruit and *A. arguta* but not *A. polygama* in commercially grown *Actinidia* spp. Each primer was carefully designed so that the nucleotides at the position corresponding to the 3′ end of the primer would be the same in all of the sequences of the target species and would differ from those of the nontarget species. Consequently, the F151 (5′-GTG ACA CTC TCA TTC CCC G-3′) and R182 (5′-TTG CAT TCT TGT TCA AGT TCC TTG A-3′) primer pair was designed for the detection of all the *Actinidia* spp., and the F123 (5′-CGG GTG TGC TCG TGT-(C) TG-3′, 5′-CGG GTG TGC TCG TGC CG-3′) and R178 (5′-CTT GTT CAA GTT CCT TGA CGC G-3′) primer pair was designed for the detection of kiwifruit and *A. arguta*.

**Specificity Analysis of Both Primer Pairs Predicted Using PCR Simulation Software.** The specificity of the primer pairs was predicted with PCR simulation software. With both primer pairs, PCR products of the expected sizes (F151 and R182, 74 bp; F123 and R178, 92 bp) were predicted from the ITS-1 sequences of the target *Actinidia* spp., which are indicated with an asterisk (*) in **Table 1**. Although products of the target size were predicted from the nontarget wild *Actinidia* spp. when the F123 and R178 primer pair was used, the primer pair successfully differentiated *A. polygama* (matatabi) from other commercially distributed *Actinidia* spp., that is, kiwifruits and sarunashi (**Table 1B**). We considered that amplification products of the target size predicted from the wild species of *Actinidia* would not cause significant problems in developing kiwifruit detection methods, because those wild species were presumed unlikely to be mixed in foods. No products were predicted for the other plants used for food (including fruits), except for some products of nontarget size predicted from melon and avocado when the primer pair of F151 and R182 was used (**Table 1A**).

**Specificity and Sensitivity of the Detection Method for All *Actinidia* Species.** The specificity of the proposed detection method using the F151 and R182 primer pair was confirmed by the PCR experiments described under Materials and Methods. As shown in **Figure 1**, a PCR product of the target size (74 bp) was amplified from the genomic DNAs extracted from *A. deliciosa* cv. Hayward, *A. chinensis* cv. Hort16A, *A. arguta* cv. Issai, *A. arguta* (baby kiwi), and *A. polygama* (matatabi). The nucleotide sequence analyses of the PCR products confirmed that the target sequences of the *Actinidia* spp. had been amplified using the designed primer pairs (data not shown). Although some nonspecific products were sporadically amplified from satsuma orange, persimmon, and orange, all of them were different in size from the target products (data not shown). Furthermore, the sensitivity of the proposed detection method was confirmed using PCR. As shown in **Figure 2**, the PCR products of the target size were detected from 50 fg of DNA of all the target *Actinidia* spp., including three interspecific hybrids in all eight replicate runs. The target products were also detected in 50 ng of salmon testis DNA spiked with 1 ppm (w/w) of Hayward DNA and in a yogurt sample spiked with 1700 ppm (w/w) of fresh kiwifruit puree, which corresponded to ca. 10 ppm of kiwifruit protein (data not shown).

**Specificity and Sensitivity of the Detection Method for Kiwifruit and *A. arguta*.** The specificity of the proposed detection method using the F123 and R178 primer pair was

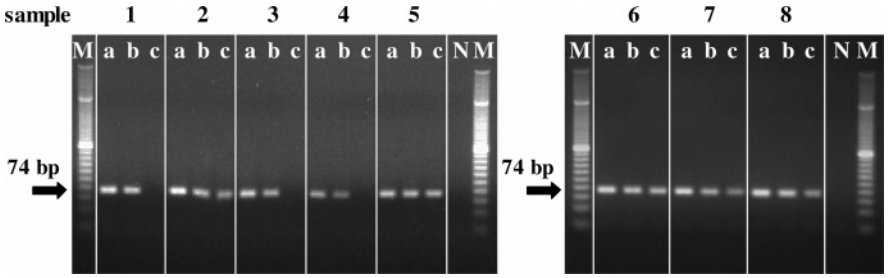


**Figure 2.** Sensitivity of the F151 and R182 primer pair for all *Actinidia* spp. The arrowhead indicates the expected PCR product. M, DNA marker (20 bp ladder, Takara Bio Inc.); N, negative control (no template). (Samples 1–8) Genomic DNA of Hayward (1), Hort16A (2), Issai (3), baby kiwi (4), matatabi (5), Kosui (6), Shinzan (7), and Sanuki gold (8). (Lanes a–c) Amplification of 500 fg (a), 50 fg (b), and 5 fg (c) of sample genomic DNA.

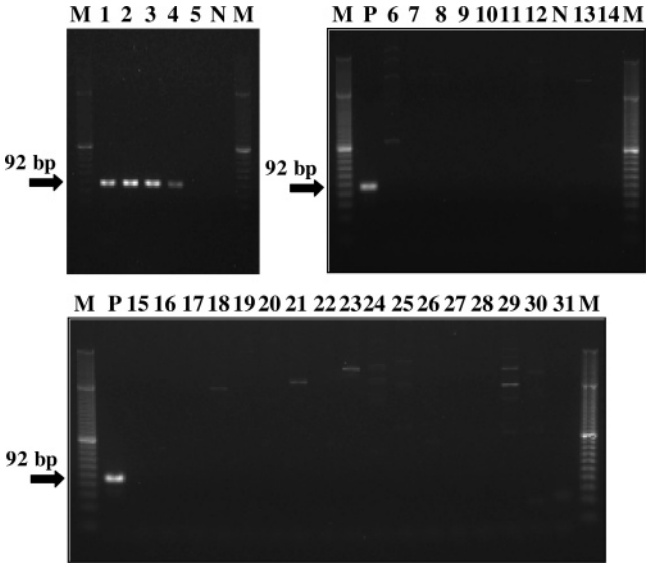


**Figure 3.** Specificity of the F123 and R178 primer pair for kiwifruit and *A. arguta* in commercially grown *Actinidia* spp. The arrowhead indicates the expected PCR product. M, DNA marker (20 bp ladder, Takara Bio Inc.); P, amplification of 500 pg of Hayward genomic DNA as a positive control; N, negative control (no template). (Lanes 1–31) Amplification of 50 ng of Hayward (1), Hort16A (2), Issai (3), baby kiwi (4), matatabi (5), corn (6), soybean (7), pear (8), strawberry (9), persimmon (10), grape (11), blueberry (12), banana (13), orange (14), peach (15), apricot (16), cherry (17), Japanese apricot (18), raspberry (19), prune (20), apple (21), papaya (22), mango (23), avocado (24), fig (25), pineapple (26), aloe (27), melon (28), satsuma orange (29), rice (30), and wheat (31) genomic DNA.

confirmed using PCR analysis. As shown in **Figure 3**, a PCR product of the target size (92 bp) was detected in the genomic DNAs extracted from *A. deliciosa* cv. Hayward, *A. chinensis* cv. Hort16A, *A. arguta* cv. Issai, and *A. arguta* (baby kiwi), but was not detected in the genomic DNA extracted from *A. polygama* (matatabi). The nucleotide sequence analyses of the PCR products confirmed that the expected sequences of the *Actinidia* spp. had been amplified (data not shown). Although nonspecific products were often amplified from some of the fruits and grains tested, all of them were different in size from the target (**Figure 3**). The sensitivity of the proposed method was also examined using PCR analysis. As shown in **Figure 4**, PCR products of the target size were amplified from 500 fg of DNA of all the target *Actinidia* spp., including three interspecific hybrids, in all eight replicate runs. The target products were also detected in 50 ng of salmon testis DNA spiked with 10 ppm (w/w) of Hayward DNA and in a yogurt sample spiked with 1700 ppm (w/w) of fresh kiwifruit puree, which corresponded to ca. 10 ppm of kiwifruit protein (data not shown).

**Analysis of Commercial Products.** A sufficient amount (20 ng/$\mu$L) of DNA for PCR was obtained only from the yogurts, the cereal products, and the cookies using Genomic-tip 20/G ($\times$1 DNA extraction scale). Less than 20 ng/$\mu$L of DNA could be obtained from the other processed food samples using the same DNA extraction method. From the samples containing kiwifruit or sarunashi (samples 1−11), amplification products could be detected using both primer pairs, except in the case of the DNAs extracted from kiwifruit jam (5a), dried kiwifruit (7a), and gummy candies (11a), as shown in **Table 2**. By increasing the size of the sample and the buffer scale ($\times$10 extraction scale), amplification products were obtained from the kiwifruit jam (5b) and the gummy candies (11b). As for the dried kiwifruit (7b), amplification products could be detected only when the samples for the DNA extraction were washed with distilled water prior to homogenization. From the samples that do not contain kiwifruit or sarunashi (samples 12−14), amplification products could not be detected using either set of primer pairs. To assess the template DNA quality, the CP03-F and CP03-R primer pair for detecting a partial region of plant chloroplast DNA was used for an internal control PCR, and the expected amplification products (124 bp) were detected in all samples tested with the exception of kiwifruit juice (9a and 9b), which nonetheless yielded a positive amplicon (74 and 92 bp) by kiwifruit PCR. On the basis of the electrophoresis analyses of the DNA extracted from the kiwifruit juice, we suggested that DNA fragmentation into approximately 100 bp (which resulted in a smear pattern) had occurred due to the processing (data not shown) and that this could have caused the negative result in the validation primer pair (CP03-F and CP03-R), which gave a longer amplicon than kiwifruit primer pairs. A validation primer pair that would give a shorter amplicon should be employed when the samples containing highly fragmentated DNA are analyzed.

## DISCUSSION

Kiwifruit is known to cause serious allergic reactions. Nishiyama et al. (*13*, *14*). reported that actinidin, the major allergenic protein, was present not only in kiwifruit (*A. deliciosa* cv. Hayward) but also in Issai (*A. arguta*) and Shinzan (an interspecific hybrid of *A. arguta* $\times$ *A. deliciosa*). In fact, IgE antibody cross-reactivity between *A. arguta* and kiwifruit (*A. deliciosa*) has also been reported (*22*). In addition, some studies have suggested that certain other kinds of kiwifruit proteins can induce allergic symptoms. Lucas et al. reported that *A. chinensis*, which contains a small amount of actinidin, exhibited cross-reactivity with the serum IgE of a patient sensitive to *A. deliciosa* and provoked allergic symptoms (*23*). Therefore, it would be

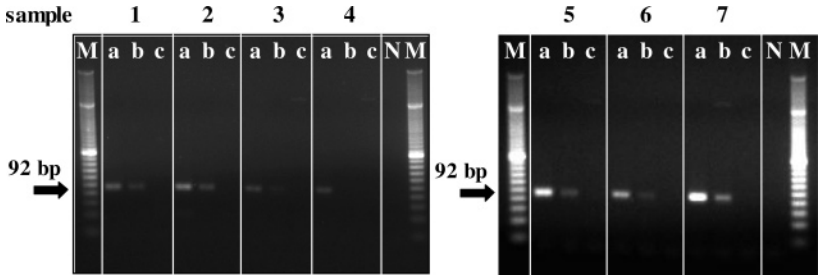


**Figure 4.** Sensitivity of the F123 and R178 primer pair for kiwifruit and *A. arguta* in commercially grown *Actinidia* spp. The arrowhead indicates the expected PCR product. M, DNA marker (20 bp ladder, Takara Bio Inc.); N, negative control (no template). (Samples 1–7) Genomic DNA of Hayward (1), Hort16A (2), Issai (3), baby kiwi (4), Kosui (5), Shinzan (6), and Sanuki gold (7). (Lanes a–c) Amplification of 500 fg (a), 50 fg (b), and 5 fg (c) of sample genomic DNA.

**Table 2.** Investigation of Commercial Products with PCR

| no. | sample | concentration of template DNA (ng/$\mu$L) | PCR results[a] CP03-F and CP03-R primer pair | F151 and R182 primer pair | F123 and R178 primer pair |
|---|---|---|---|---|---|
| commercial products containing kiwifruit or sarunashi | | | | | |
| 1 | yogurt with mixed fruit pieces | 20 | + | + | + |
| 2 | yogurt with kiwifruit pieces only | 20 | + | + | + |
| 3 | cereal with dried fruit mix | 20 | + | + | + |
| 4 | kiwifruit cookie | 20 | + | + | + |
| 5a | kiwifruit jam (×1) | <10 | + | − | − |
| 5b | kiwifruit jam (×10) | 20 | + | + | + |
| 6 | sarunashi jam | <10 | + | + | + |
| 7a | dried kiwifruit | <10 | + | − | − |
| 7b | dried kiwifruit (washing) | <10 | + | + | + |
| 8 | fruit drink mixed fruits including kiwifruit | 11 | + | + | + |
| 9a | 100% kiwifruit juice (×1) | <10 | ± | + | + |
| 9b | 100% kiwifruit juice (×10) | 20 | − | + | − |
| 10 | 10% sarunashi juice | <10 | + | + | + |
| 11a | gummy candies (×1) | <10 | + | − | − |
| 11b | gummy candies (×10) | <10 | + | ± | − |
| commercial products whthout kiwifruit or sarunashi in the list of ingredients | | | | | |
| 12 | cereal with dried fruit mix (not including kiwifruit) | 20 | + | − | − |
| 13 | cookie with grapefruit jam (not including kiwifruit) | 20 | + | − | − |
| 14 | fruit and vegetable drink (not including kiwifruit) | 15 | + | − | − |

[a] Two independent PCR on a DNA preparation from each sample: +, 2/2; ±, 1/2; −, 0/2.

desirable for patients with kiwifruit allergy to avoid consuming such kiwifruits and their relatives. Although cross-reactivity between *A. polygama* and kiwifruit has not been reported because of the limited consumption of *A. polygama*, all *Actinidia* spp. may to some extent be potentially allergenic. Therefore, we first designed a primer pair to detect all *Actinidia* spp. On the other hand, among the edible *Actinidia* spp. distributed in the Japanese market, the fruit of *A. polygama* is easily distinguishable from the others by its appearance. Moreover, as mentioned above, the allergenicity or cross-reactivity of *A. polygama* cannot be excluded entirely. Therefore, we designed another primer pair that detects both kiwifruit and *A. arguta* but not *A. polygama* among commercially distributed *Actinidia* spp.

The ITS regions used in this study should be useful sequences not only for the genus-specific detection of all the *Actinidia* spp. but also for the detection of certain selected species from among the *Actinidia* spp. The results of PCR simulation with the designed primer pairs showed that products of the target sizes were predicted from the target species.

The specificities of the PCR methods examined with the materials purchased from the market were consistent with those predicted by the PCR simulations. Although some unexpected PCR products were amplified from some fruits and grains, their sizes were clearly different from that of the target. Although these simulation results are not definitive, they should give us a good indication of the specificity of the designed primer pairs. The results of the PCR simulation and the actual PCR suggested that these methods were specific enough to detect the genomic DNA extracted from kiwifruit and the target *Actinidia* spp.

Sensitivity studies showed that, with both primer pairs, the expected PCR products were detectable from a salmon testis DNA spiked with 50−500 fg of DNA of *Actinidia* spp., as low as 1−10 ppm (w/w) of kiwifruit DNA, or a yogurt sample spiked with 1700 ppm (w/w) of fresh kiwifruit puree (corresponding to ca. 10 ppm of kiwifruit protein). In terms of sensitivity, this level was comparable to that of the buckwheat detection method developed previously (*20*). Thus, the primer pairs designed in this work could provide reliable, specific, and sensitive detection of the presence of kiwifruit and other potentially allergenic kiwifruit-related plant species. Quantification of the kiwifruit amount might also be possible when a proper internal standard is used in real-time PCR (*24*).

The applicability of the proposed methods was assessed by analyzing several commercial products containing processed kiwifruit. All tested samples except for the gummy candies gave products of the target size with both primer pairs. The gummy candies were fruit-flavored gummies that included kiwifruit flavor. The ingredient list stated that the product contained kiwifruit juice as a colorant. Therefore, we concluded that the

absence of amplified PCR products from the candies was probably due to the limited amount of kiwifruit DNA in the gummy candies.

Only the yogurts, cereals, and cookies yielded more than 20 ng/$\mu$L DNA. Because these products would contain a large quantity of the DNA originally present in the respective raw materials (milk, lactic acid bacteria, cereal grains, and so forth), most of the DNA yielded from these products was mainly attributable to the matrix materials. On the other hand, the amounts of DNA extracted from jams and juices containing either kiwifruit or sarunashi (*A. arguta*) and from dried kiwifruit were lower than expected, even if large-scale DNA extractions were performed. Some improvements were made in the detection of kiwifruit from the kiwifruit jam and the gummy candies by scaling up the sample size of DNA extraction or washing the dried kiwifruit with distilled water prior to the homogenization for DNA extraction. Further studies are underway to improve the DNA extraction and the interlaboratory validation of the methods using processed food models that contain a known amount of kiwifruit.

In conclusion, we designed two kiwifruit detection primer pairs and developed a highly sensitive and specific PCR method of kiwifruit detection using these primer pairs. These methods would be expected to be useful for detecting kiwifruit in processed foods to confirm the validity of food labeling and to ensure the safety of allergic patients.

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