Data Article

Data representing applicability of developed growth hormone 1 (GH1) gene detection method for detecting Atlantic salmon (Salmo salar) at high specificity to processed salmon commodities

Keisuke Soga a, Kosuke Nakamura a,*, Takumi Ishigaki a, Shinya Kimata a, Kiyomi Ohmori b, Masahiro Kishine c, Junichi Mano c, Reona Takabatake c, Kazumi Kitt a, Hiroyuki Nagoya d, Kazunari Kondo a

a National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 215-9501, Japan
b Chemistry Division, Kanagawa Prefectural Institute of Public Health, 1-3-1 Shimomachiya, Chigasaki, Kanagawa, 253-0087, Japan
c Division of Analytical Science, Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki, 305–8642, Japan
d Research Center for Aquatic Breeding, National Research Institute of Aquaculture, Fisheries Research and Education Agency, 224-1 Hiruta, Tamaki-cho, Mie, 519-0423, Japan

ABSTRACT

This article is referred to the research article entitled “Development of a novel method for specific detection of genetically modified Atlantic salmon, AquAdvantage, using real-time polymerase chain reaction” by Soga et al. (2020). Applicability of the developed growth hormone 1 (GH1) and 18S ribosomal DNA (18S rDNA) detection methods using real-time polymerase chain reaction (PCR) for detecting Atlantic salmon (Salmo salar) to processed food commodities was examined. DNAs extracted and purified from 24 commodities labelled to include salmon as an ingredient were used as template. Yield and purity of
Real-time polymerase chain reaction (PCR)
Growth hormone 1
Processed foods
Detection method

DNA samples were quantified by measuring the UV absorption at 260 nm (A260) using ND-1000 spectrophotometer. The quality of the samples was estimated from the UV absorption ratios at 260 and 280 nm (A260/A280) and 260 and 230 nm (A260/A230). For real-time PCR analysis, the baseline was set to cycles 3 through 15. The ΔRn threshold for plotting the cycle threshold (Cq) values was set to 0.2 during exponential amplification. Reactions with the Cq value of less than 48 and exponential amplification plots were scored as positive. If the Cq value could not be obtained, the reaction was scored as negative. Reactions with the Cq value of less than 48, but without exponential amplification as judged by visual inspection of the respective ΔRn plots and multi-component plots were scored as negative.

Data source location
Institution: National Institute of Health Sciences
City/Town: Kawasaki/Kanagawa
Country: Japan

Data accessibility
With the article

Related research article
Author's name: Keisuke Soga, Kosuke Nakamura, Takumi Ishigaki, Shinya Kimata, Kiyomi Ohmori, Masahiro Kishine, Junichi Mano, Reona Takabatake, Kazumi Kitta, Hiroyuki Nagoya, Kazunari Kondo
Title: Development of a novel method for specific detection of genetically modified Atlantic salmon, AquAdvantage, using real-time polymerase chain reaction
Journal: Food Chemistry
DOI: https://doi.org/10.1016/j.foodchem.2019.125426

Value of the Data
- These data provide information concerning applicability of GH1 and 18S rDNA detection methods to various processed salmon commodities to detect Salmo salar (Atlantic salmon) ingredient.
- The data are beneficial for researchers who are trying to detect or confirm the presence of Atlantic salmon derived ingredient in foods.
- These data show potential of developed real-time PCR detection method to monitor Atlantic salmon in various processed salmon commodities at high sensitivity and specificity.
- The data provide information concerning DNA yield and purification efficiency from various processed salmon commodities that were produced worldwide and applicability of developed GH1 detection method [1] that was more sensitive than the original method described [2] to the commodities.
1. Data

Table 1 summarizes the information on processed salmon commodities, including food processing type, production country, food ingredient labelled in Japan and recommended preservation state. Table 2 is the list of information on sequences of real-time PCR primer pairs and probes used in this dataset. Table 3 presents the specificity test data using developed 18S rDNA detection method. Table 4 shows the data of DNA yields and purity obtained from 24 kinds of processed salmon commodities, and of real-time PCR tests using 18S rDNA and GH1 detection methods.

| Sample no. | Food processing type | Commodity name | Production country | Ingredient on the food label in Japan | Recommended preservation state |
|------------|----------------------|----------------|--------------------|--------------------------------------|--------------------------------|
| 1          | Smoked               | Smoked salmon  | Chile              | Atlantic salmon, Salt, Sugar         | Frozen                         |
| 2          | Smoked               | Smoked salmon  | Japan              | Chilean cultured Atlantic salmon, Salt, Sugar, Spice | Frozen                         |
| 3          | Smoked               | Smoked salmon  | Japan              | Norwegian Atlantic salmon, Seaweed salt, White superior soft sugar, Onion powder, (A part of the ingredients contains a salmon.) | Frozen                         |
| 4          | Sliced               | Atlantic salmon baked slice | Vietnam | Norwegian cultured Atlantic salmon | Frozen                         |
| 5          | Daily dish           | Daily dish     | Japan              | Atlantic salmon                      | Frozen                         |
| 6          | Smoked salmon slice  | Vietnam        | Norwegian Atlantic salmon | Frozen                         |
| 7          | Canned               | Atlantic salmon backbone boiled in water | Vietnam | Norwegian Atlantic salmon, Salt | Room temperature |
| 8          | Processed salmon     | Thailand        | Atlantic salmon, Soybean oil, Salt, Garlic paste, Vegetables extract, Spice/Seasoning (Amino acids etc.), Thickener (Guar Gum) | Room temperature |
| 9          | Processed fish       | France          | Atlantic salmon, Food with milk etc., Smoked Atlantic salmon, Wheat flour, Tomato paste, Mustard, Processed milk protein, Salt, Pepper, Allspice, Coriander | Room temperature |
| 10         | Salmon midrib boiled in water | Japan | Silver salmon midrib (Oncorhynchus kisutch), Salt | Room temperature |
| 11         | Smoked Atlantic salmon with oil | Japan | Atlantic salmon, Soybean oil, Salt, Sake | Room temperature |
| 12         | Pink salmon boiled in water | Japan | Pink salmon (Oncorhynchus gorbuscha), Salt | Room temperature |
| 13         | Dried Fishery dried products | Japan | Norwegian Atlantic salmon, Chilean Rainbow trout (Oncorhynchus mykiss), Rice vinegar, Seasoning (Amino acids etc.), (A part of the ingredients contains soybean and wheat.) | Room temperature |
| 14         | Salmon flakes        | Japan           | Chum salmon (Oncorhynchus keta), Vegetable fats and oils (Rapeseed oil, Soybean oil), Reduced sugar syrup, Salt, Bonito extract powder, Kelp extract/Trehalose, Seasoning (Amino acids etc.), Antioxidant (Vitamin E), Emulsifier, Colorants (Red 102, Yellow 5), (A part of the ingredients contains salmon, soybean and fish sauce.) | Room temperature |
| 15         | Salmon flakes        | Japan           | Chum salmon (Oncorhynchus keta), Vegetable fats and oils, Sugar, Salt, Reduced sugar syrup/Seasoning (Inorganic salt etc.), Colorants (Monascus, Carotinoid), (A part of the ingredients contains salmon and soybean,) | Room temperature |
| 16         | Pasta sauce          | Japan           | Salmon sauce (Shortening [Rapeseed oil, Palm oil, Palm kernel oil]), Atlantic Salmon, Dextrin, Salt, Seafood extract, Sugar, Dried bonito | Room temperature |

(continued on next page)
2. Experimental design, materials, and methods

Twenty-four processed salmon commodities including six types (smoked, sliced, canned, dried, roe and pickled) were purchased through the Internet in Japan. Details of food ingredients labelled, production country and recommended preservation state were described in Table 1. The commodities were selected taking into account that a wide range of salmonids species is covered in the test, based on the phylogeny identified in salmonid species [3].

Sampling weight was 1 g for smoked, sliced, canned, roe and pickled commodities, and 0.5 g for dried commodities. DNA extraction and purification were done using GM quicker 3 kit (Nippon gene, Toyama, Japan) for smoked, sliced, canned and dried commodities. Genomic-tip 20/G (Qiagen, Hilden,
Germany) was used for salmon roe and pickled commodities. The quantity and the quality of DNA obtained were estimated from the ultraviolet (UV) absorption at 260 nm and its ratios at 260 and 280 nm (A$_{260}$/A$_{280}$) and 260 and 230 nm (A$_{260}$/A$_{230}$), respectively, using ND-1000 spectrophotometer (Thermo Fisher Scientific).

The primer pair and probe targeting GH1 were used as described previously [1,2]. The primer pair and probe targeting 18S rDNA were designed using Primer Express Software (Thermo Fisher Scientific, Version 3.0.1), based on the sequence given by NCBI (Salmo salar 18S ribosomal RNA gene, partial sequence, GenBank accession no. FJ710886) (Table 2). The probes were labelled with 6-carboxyfluorescein (FAM) at 5’ terminus and with 6-carboxytetramethylrhodamine (TAMRA) at 3’

Table 2
List of oligonucleotide primers and probes used.

| Detection method | Target region (GenBank accession no.) | Name | Nucleotide sequences (5’ → 3’)$^a$ | Amplicon (bp) | Reference |
|------------------|--------------------------------------|------|----------------------------------|--------------|-----------|
| GH1              | Growth hormone 1 (X61938)            | AM5F | AAGGTGCAAAAAACCATGTTGCTTCT       | 176          | [1]       |
|                  |                                      | AM5R | ATGTGACGTCTCTAGGTCTAGAC          |              |           |
|                  |                                      | AM5PR-2 | [FAM]TTGGTTTCTTGTGCTTCT         |              |           |
|                  |                                      |      | ATTGCAGAAGTA[TAMRA]             |              |           |
| 18S rDNA         | 18S ribosomal DNA (FJ710886)         | 18S-F | TGGCCGCTAGAGGTGAAATT             | 61           | This dataset |
|                  |                                      | 18S-R | GCAATAATCCTTCCGCTTCTCG          |              |           |
|                  |                                      | 18S-P | [FAM]TTGGACCGGC                 |              |           |
|                  |                                      |      | GCAAGACCG[TAMRA]                |              |           |

$^a$ FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

The primer pair and probe targeting GH1 were used as described previously [1,2]. The primer pair and probe targeting 18S rDNA were designed using Primer Express Software (Thermo Fisher Scientific, Version 3.0.1), based on the sequence given by NCBI (Salmo salar 18S ribosomal RNA gene, partial sequence, GenBank accession no. FJ710886) (Table 2). The probes were labelled with 6-carboxyfluorescein (FAM) at 5’ terminus and with 6-carboxytetramethylrhodamine (TAMRA) at 3’

Table 3
Specificity test for 18S rDNA real-time PCR method.

| Organism name | NCBI taxonomy ID | Real-time PCR detection$^a$ |
|---------------|------------------|-----------------------------|
| Plants        |                  |                             |
| Allium cepa L.| 4679             | –                           | –                           |
| Apium graveolens | 117781       | –                           | –                           |
| Arachis hypogaea L. | 3818     | –                           | –                           |
| Brassica napus L. | 3708         | –                           | –                           |
| Brassica rapa var. perviridis | 344680 | –                           | –                           |
| Capsicum annuum var. annuum | 40321 | –                           | –                           |
| Carica papaya L. | 3649          | –                           | –                           |
| Citrus limon (L.) Burm.f. | 2708 | –                           | –                           |
| Cucurbita L. | 3660             | –                           | –                           |
| Daucus carota L. | 4039          | –                           | –                           |
| Glycine max (L.) Merr. | 3847 | –                           | –                           |
| Oryza sativa L. | 4530            | –                           | –                           |
| Solarium lycopersicum | 4081 | –                           | –                           |
| Solarium melongena L. | 4111         | –                           | –                           |
| Solarium tuberosum L. | 4113         | –                           | –                           |
| Triticum aestivum L. | 4565 | –                           | –                           |
| Zea mays L. | 4577             | –                           | –                           |
| Animals       |                  |                             |
| Bos taurus    | 9913             | 40.80                       | 40.56                       |
| Sus scrofa    | 9823             | 40.54                       | 42.18                       |
| Fishes        |                  |                             |
| Hyperoglyphe japonica | 171196 | 18.28                       | 18.32                       |
| Oncorhynchus keta | 8018          | 19.73                       | 19.63                       |
| Oncorhynchus kisutch | 8019         | 19.59                       | 19.48                       |
| Oncorhynchus masou ishikawae | 8021     | 20.68                       | 20.69                       |
| Oncorhynchus mykiss | 8022         | 20.45                       | 20.51                       |
| Pagrus major  | 143350           | 19.01                       | 19.01                       |
| Scomber japonicus | 13676        | 18.70                       | 18.73                       |
| Salmo salar (Atlantic salmon) | 8030 | 19.53                       | 19.71                       |
| No template control | –             | –                           | –                           |

$^a$ Cq values obtained from a duplicate test per a sample were indicated.

$^-$. The reaction was scored as negative.
terminus. Oligonucleotide sequences of primer pairs and probes used in this dataset are shown in Table 2.

Fifty nanograms of extracted and purified DNA were used as template for real-time PCR analysis in duplicate tests per a sample. The fluorescence intensity when amplifying targeted DNA sequences was monitored by ABI PRISM 7900 Sequence Detection System (Thermo Fisher Scientific). Thermal cycling conditions were 95 °C for 10 min, followed by 50 cycles of 15 sec at 95 °C and 1 min at 57 °C. The baseline was set to cycles 3 through 15. The ΔRn threshold for plotting the cycle threshold (Cq) values was set to 0.2 during exponential amplification.

For tests, reactions with the Cq value of less than 48 and exponential amplification plots were scored as positive. If the Cq value was more than 48 or could not be obtained, the reaction was scored as negative. Reactions with the Cq value of less than 48, but without exponential amplification as judged by visual inspection of the respective ΔRn plots and multi-component plots were scored as negative. Specificity test of 18S rDNA method was performed using 17 kinds of plants, 2 kinds of animals and 8 kinds of fishes (Table 3).

Acknowledgments

This project was conducted under the support of the Ministry of Health, Labour and Welfare of Japan.

Table 4
DNA yield and purity obtained from the processed salmon commodities.

| Sample No. | Salmon type | DNA yield (ng/g)a | DNA purity | Real-time PCR detectionb | 18S rDNA method | GH1 method |
|------------|-------------|------------------|------------|--------------------------|-----------------|------------|
|            |             | A260/A280c | A260/A230d |                          |                 |            |
| 1          | Salmo salar | 87,375      | 1.53      | 2.77                     | 17.03           | 16.90      | 27.49      | 27.38      |
| 2          | (Atlantic salmon) | 95,125   | 1.56      | 2.73                     | 16.81           | 16.71      | 27.71      | 27.65      |
| 3          | 63,875      | 1.50      | 2.79      | 17.03                   | 16.99           | 27.22      | 27.12      |
| 4          | 64,625      | 1.50      | 2.77      | 16.51                   | 16.45           | 27.13      | 27.08      |
| 5          | 120,600     | 1.53      | 2.68      | 16.99                   | 16.96           | 27.60      | 27.53      |
| 6          | 101,800     | 1.55      | 2.61      | 17.12                   | 16.99           | 27.90      | 28.03      |
| 7          | 40,700      | 1.56      | 2.48      | 19.96                   | 20.01           | 35.12      | 35.92      |
| 8          | 84,400      | 1.52      | 2.64      | 18.98                   | 18.99           | 31.16      | 31.27      |
| 9          | 48,250      | 1.49      | 2.90      | 19.05                   | 19.16           | 32.48      | 32.63      |
| 11         | 158,250     | 1.52      | 2.72      | 19.42                   | 19.45           | 32.92      | 32.92      |
| 13         | 325,000     | 1.45      | 2.70      | 19.55                   | 19.49           | 31.35      | 31.50      |
| 16         | 38,750      | 1.48      | 2.99      | 18.06                   | 17.97           | 27.42      | 27.37      |
| 24         | 89,500      | 1.62      | 2.64      | 18.44                   | 18.89           | 28.35      | 28.79      |
| 10         | Oncorhynchus kisutch | 68,250    | 1.46      | 3.05                     | 21.24           | 21.30      | –          | –          |
| 12         | Oncorhynchus gorbuscha | 171,250 | 1.53      | 2.70                     | 19.34           | 19.29      | –          | –          |
| 14         | Oncorhynchus keta | 143,500   | 1.46      | 2.79                     | 19.26           | 19.11      | –          | –          |
| 15         | 146,000     | 1.45      | 2.76      | 21.03                   | 20.84           | –          | –          |
| 22         | Oncorhynchus nerka | 47,200   | 1.56      | 2.64                     | 19.30           | 19.12      | –          | –          |
| 17         | Unknown     | 2,063     | 1.51      | 1.33                     | 24.76           | 24.85      | –          | 42.14      |
| 18         | 56,400      | 1.49      | 2.21      | 20.53                   | 20.22           | –          | –          |
| 19         | 84,000      | 1.55      | 2.62      | 19.00                   | 18.80           | –          | –          |
| 20         | 20,400      | 1.47      | 2.64      | 20.28                   | 20.43           | –          | –          |
| 21         | 20,500      | 1.45      | 2.71      | 19.42                   | 19.43           | –          | –          |
| 23         | 16,200      | 1.49      | 2.63      | 22.66                   | 22.39           | –          | –          |

a The reaction was scored as negative.
b According to the information on food label.
c DNA yield (ng) per 1 g sample.
d Absorbance ratio at 260 and 280 nm.
e Absorbance ratio at 260 and 230 nm.
f Cq values obtained from a duplicate test per a sample were indicated.
Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] K. Soga, K. Nakamura, T. Ishigaki, S. Kimata, K. Ohmori, M. Kishine, J. Mano, R. Takabatake, K. Kitta, H. Nagoya, K. Kondo, Development of a novel method for specific detection of genetically modified Atlantic salmon, AquAdvantage, using real-time polymerase chain reaction, Food Chem. 305 (2020) 125426, https://doi.org/10.1016/j.foodchem.2019.125426.

[2] A.B. Hafsa, N. Nabi, M.S. Zellama, K. Said, M. Chaouachi, A new specific reference gene based on growth hormone gene (GH1) used for detection and relative quantification of Aquadvantage® GM salmon (Salmo salar L.) in food products, Food Chem. 190 (2016) 1040–1045, https://doi.org/10.1016/j.foodchem.2015.06.064.

[3] S. Murata, N. Takasaki, M. Saitoh, H. Tachida, N. Okada, Details of retropositional genome dynamics that provide a rationale for a generic division: the distinct branching of all the Pacific salmon and Trout (Oncorhynchus) from the Atlantic salmon and trout (Salmo), Genetics 142 (1996) 915–926.