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Development of a novel method for specific detection of genetically modified Atlantic salmon, AquAdvantage, using real-time polymerase chain reaction

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ABSTRACT

Genetically modified (GM) Atlantic salmon, AquAdvantage (AquAd), was the first GM animal approved officially for human consumption. Many countries monitor the use of this product under their GM regulations, but a pragmatic system for AquAd-specific detection is needed. Here, we developed a real-time polymerase chain reaction method with high sensitivity for detection of AquAd in foods. This method showed high specificity for the AquAd transgene and the detection limit was 12.5–25 targeted DNA copies per test reaction. An inter-laboratory study using the method developed demonstrated reproducibility at > 0.1% (w/w) AquAd content.

1. Introduction

Salmon aquaculture is one of the fastest growing food production systems in the world (FAO, 2018). A genetically modified (GM) *Salmo salar* (Atlantic salmon, NCBI taxonomy ID 8030), 'AquAdvantage' (hereinafter referred to as AquAd), was developed to be more efficient for aquaculture compared with conventional non-GM Atlantic salmon (AquaBounty Technologies, Inc., 2010). Sponsored by AquaBounty Technologies Inc., AquAd was approved for human consumption by the United States in 2015 (Ledford, 2015; Waltz, 2016) and by Canada in 2016 (Waltz, 2017). However, use of AquAd as a food in other countries requires appropriate national or regional (e.g. European Union [EU]) approval. Consequently, a monitoring system to promote control of AquAd for foods is needed, which requires a specific and sensitive detection method.

AquAd was developed by inserting the opAFP-GHc2 transgene construct (EO-1α) into the Atlantic salmon's genomic DNA, which includes a single copy transgene cassette coding a Chinook salmon-derived growth hormone gene driven by an *antifreeze protein* promoter from the ocean pout (Du et al., 1992; Yaskowiak, Shears, Agarwal-Mawal, & Fletcher, 2006). AquAd for human consumption is a triploid female Atlantic salmon that is hemizygous for the transgene construct (AquaBounty Technologies, Inc., 2010). Detection methods for AquAd, based on the transgene construct, have been developed previously (Castro, Amorim, Moreira, & Pereira, 2017; Debode et al., 2018). However, for the purpose of GM regulation, evidence of AquAd presence in food products is also required and, to identify the presence of AquAd, a detection method targeting the unique genomic DNA sequences flanking the transgene integration site is needed to distinguish it from the other transgenic species.

In this study, a novel AquAd-specific detection method was developed for use in foods and an inter-laboratory study was conducted to validate this method.

2. Materials and methods

2.1. Food samples

AquAd and non-GM Atlantic salmon fillets were kindly provided by AquaBounty Technologies, Inc. (Maynard, MA, USA). Seven other fish

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Table 1
List of oligonucleotide primers and probes used in this study.

Detection method	Target region	Name	Nucleotide sequences (5′→3′)*	Amplicon (bp)	Reference
AquAd	AquAdvantage (event-specific)	AquAd-F	TGCTGATGCCTCTGATACCAC	156	This study
		AquAd-R	ATGCCTCTAGTGCAAGTTCAGTC		
		AquAd-P	[FAM]CAGTAGTACAACGTTGGCAGATGTATGAGAACT[BHQ1]		
GH1 (endogenous gene)	*Growth hormone 1* (GenBank Accession No. X61938)	AM5F	AAGGTGCAAAACCATGTTGCCTTCT	176	Hafsa, et al. and this study
		AM5R	ATGTGAGTGTTCTAGGTCACTAGAC		
		AM5PR-2	[FAM]TGCGTTTCTTGTGCTCTCTATTGCAAAGTA[TAMRA]		

*FAM, 6-carboxyfluorescein; BHQ1, black hole quencher 1; TAMRA, 6-carboxytetramethylrhodamine.

species (*Hyperoglyphe japonica* [NCBI taxonomy ID 171196], *Oncorhynchus keta* [NCBI taxonomy ID 8018], *Oncorhynchus kisutch* [NCBI taxonomy ID 8019], *Oncorhynchus masou ishikawae* [NCBI taxonomy ID 8021], *Oncorhynchus mykiss* [NCBI taxonomy ID 8022], *Pagrus major* [NCBI taxonomy ID 143350] and *Scomber japonicus* [NCBI taxonomy ID 13676]), two animals (*Bos taurus* [NCBI taxonomy ID 9913] and *Sus scrofa* [NCBI taxonomy ID 9823]) and 17 plant species (*Allium cepa* L. [NCBI taxonomy ID 4679], *Apium graveolens* Dulce Group [NCBI taxonomy ID 117781], *Arachis hypogaea* L [NCBI taxonomy ID 3818], *Brassica napus* L. [NCBI taxonomy ID 3708], *Brassica rapa* var. *perviridis* [NCBI taxonomy ID 344680], *Capsicum annuum* var. *annuum* [NCBI taxonomy ID 40321], *Carica papaya* L. [NCBI taxonomy ID 3649], *Citrus limon* (L.) *Burm.f.* [NCBI taxonomy ID 2708], *Cucurbita* L. [NCBI taxonomy ID 3660], *Daucus carota* L. [NCBI taxonomy ID 4039], *Glycine* max (L.) *Merr.* [NCBI taxonomy ID 3847], *Oryza sativa* L. [NCBI taxonomy ID 4530], *Solanum lycopersicum* [NCBI taxonomy ID 4081], *Solanum melongena* L. [NCBI taxonomy ID 4111], *Solanum tuberosum* L. [NCBI taxonomy ID 4113], *Triticum aestivum* L. [NCBI taxonomy ID 4565] and *Zea mays* L. [NCBI taxonomy ID 4577]) were purchased from supermarkets in Japan (Table 2). One from each species was obtained and tested in this study.

2.2. DNA extraction and purification

All samples for DNA preparation were pooled per each sample or product package and pulped using a food processor. DNA was extracted and purified from five grams of pulped sample using an ion-exchange resin-type DNA extraction and purification kit (Genomic-tip 100/G; Qiagen, Hilden, Germany). The protocol provided by the manufacturer was modified slightly as follows: 30 mL G2 buffer (Qiagen) containing 800 mM guanidine HCl, 30 mM Tris HCl (pH 8.0), 30 mM EDTA (pH 8.0), 5% (v/v) Tween-20 and 0.5% (v/v) Triton X-100, 20 μL 100 mg/mL RNase A (Qiagen) and 500 μL cellulase (for plant samples only, Sigma-Aldrich, St. Louis, MO, USA) were added to the pulped sample and mixed thoroughly using a vortex mixer. Plant samples were incubated at 50 °C for an hour for digestion by cellulases. Proteinase K (200 μL; Promega, Madison, WI, USA) was added and the mixture was incubated at 50 °C for another hour, during which time the tubes were regularly inverted to mix the contents. The samples were centrifuged at 3000 × *g* for 20 min at 4 °C and the supernatants applied to a Genomic-tip 100/G column (Qiagen) that was pre-equilibrated with 4 mL QBT buffer (Qiagen) containing 740 mM NaCl, 50 mM 3-(*N*-morpholino) propanesulfonic acid (pH 7.0), 15% (v/v) isopropanol and 0.15% (v/v) Triton X-100. The column was washed three times with 7.5 mL QC buffer (Qiagen) containing 1 M NaCl, 50 mM MOPS (pH 7.0) and 15% (v/v) isopropanol, and transferred to a centrifuge tube. Then, an equal volume of isopropyl alcohol was added, and the sample was mixed thoroughly. DNA was collected by centrifugation at 12,000 × *g* for 15 min. The pellet was rinsed with 1 mL 70% (v/v) ethanol and centrifuged at 12,000 × *g* for 5 min. The supernatant was removed using an aspirator, and the DNA precipitate was dissolved in 50 μL pure water.

The concentration and quality of DNA samples were estimated by measuring UV absorption at 230 nm (A_{230}), 260 nm (A_{260}) and 280 nm (A_{280}) using an ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The quality of samples was estimated from the UV absorption ratios (A_{260}/A_{230} and A_{260}/A_{280}). The DNA samples were diluted to 10 ng/μL using pure water before being used as real-time PCR templates.

2.3. Oligonucleotide primers and probes

The real-time PCR primers and probe were designed to hybridize AquAd event-specific genomic sequences based on the sequence described in the previous report (Yaskowiak et al., 2006), using Primer Express Software Version 3.0.1 (Thermo Fisher Scientific Inc). Primers and probes for *Growth hormone gene 1* (*GH1*), an endogenous salmon-specific gene, were prepared as reference controls, based on Hafsa, Nabi, Zellama, Said, and Chaouachi (2016) with slight modifications. The oligonucleotide probe, AquAd-P, was labeled with 6-carboxyfluorescein (FAM) at the 5′ terminus and a black hole quencher 1 (BHQ1) was added at the 3′ terminus; AM5PR-2 was labeled with FAM at the 5′ terminus and 6-carboxytetramethylrhodamine (TAMRA) was added at the 3′ terminus. The oligonucleotide sequences are listed in Table 1. All primers and fluorescent dye-labeled probes were synthesized by Eurofins Genomics (Tokyo, Japan), diluted with pure water, and stored at −20 °C until use

2.4. Real-time PCR

Real-time PCR was performed using an ABI PRISM 7900 Sequence Detection System (7900HT) (Thermo Fisher Scientific Inc). Five microliters of the DNA solution (containing 50 ng DNA) were mixed with 12.5 μL FastStart Universal Probe Master (ROX) (Roche Diagnostics, Basel, Switzerland), 0.8 μM of each primer, and 0.1 μM probe in a final volume of 25 μL. The PCR conditions were 95 °C for 10 min, followed by 50 cycles of 15 *sec* at 95 °C and 1 min at 57 °C. Non-GM salmon genomic DNA was used as no template controls (NTC).

Results were analyzed using SDS 2.4 sequence detection software (Thermo Fisher Scientific Inc). The normalized reporter signal (ΔRn) threshold for plotting cycle threshold (Cq) values was set to 0.2. Reactions with a Cq value of < 48 and exponential amplification, as judged by visual inspection of the respective ΔRn plots and multi-component plots, were scored as positive. If a Cq value could not be obtained or was $\geqq 48$, the reaction was scored as negative. Reactions with a Cq value of < 48, but without exponential amplification, as judged by visual inspection, were also scored as negative. For specificity test, real-time PCR was performed in duplicate for each genomic DNA sample.

2.5. Limit of detection (LOD) test

An LOD test was performed using a control plasmid that contained the real-time PCR targeted DNA sequences. Plasmid DNA was serially-diluted using 10 ng/μL non-GM salmon genomic DNA solution to concentrations of 6.25, 12.5, 25, 50, 100 and 1000 copies per 5 μL. The LOD test was performed by repeating real-time PCR 21 times per sample.

2.6. Inter-laboratory validation

An inter-laboratory validation of the method was performed by three independent laboratories that own a 7900HT system. The study was organized by the National Institute of Health Sciences according to guidelines for qualitative real-time PCR methods (Broeders et al., 2014; BVL, 2016b). According to a procedure described previously (Thompson, Ellison, & Wood, 2006), homogeneity of the test samples was verified before dispatch to participating laboratories. Ten test samples of each DNA concentration (0%, 0.1%, 0.5% and 1% [w/w] that were diluted using non-GM salmon genomic DNA solution) were labeled with randomized numbers that were generated by Excel software (Microsoft Co, Redmond, WA, USA), and tested in duplicate for homogeneity. Each blind sample was tested to determine Cq values at the threshold value (0.2) from exponential amplification plots obtained from the real-time PCR.

Data were analyzed by Cochran's test and one-way analysis of variance. Each laboratory received six lots of each sample concentration, as blind samples, and performed the qualitative AquAd method using their 7900HT system. Each sample was tested in duplicate. If both results were positive, the sample was scored as a positive. If either or all results were negative, the sample was scored as a negative.

3. Results

3.1. Evaluation of real-time PCR primers and probe sets

To detect AquAd specifically, a real-time PCR primers and probe set was designed based on AquAd-specific genomic DNA sequences. As shown in Fig. 1, the targeted DNA sequences were at a junction between salmon genomic DNA and the 5′ terminus of the antifreeze protein gene promoter derived from *Ocean pout* (Yaskowiak et al., 2006). As a reference control, we used a real-time PCR primers and probe set targeting the endogenous Atlantic salmon gene, *GH1*, as described previously (Hafsa et al., 2016). These methods for detecting AquAd-specific DNA sequences and *GH1* sequences were named AquAd and GH1 detection methods, respectively. DNA sequences of the oligonucleotides used are listed in Table 1.

To test the specificity of AquAd and GH1 detection methods, genomic DNA samples purified from 27 other food products, including fruits, grains, vegetables, animals and fish, as well as AquAd, were used as real-time PCR templates. Using the AquAd detection method, no positive amplification signals were observed from any samples except when AquAd genomic DNA was present (Fig. 2A). Fifty nanograms of AquAd template DNA generated Cq values of 29.64/29.51 from duplicate tests using the AquAd detection method (Table 2). No positive signals were detected by testing duplicate using other template DNA prepared from crops, animals or fish, including other Salmonidae species (i.e. *Oncorhynchus keta*, *Oncorhynchus kisutch*, *Oncorhynchus masou ishikawae* and *Oncorhynchus mykiss*).

Using the equivalent DNA templates, the GH1 detection method was tested, using the primers and probe shown in Table 1. As shown in Fig. 2B, the Atlantic salmon derived DNA was amplified successfully, but those of other fish were not, indicating that the method is specific to Atlantic salmon genomic DNA.

3.2. Sensitivity of the AquAd detection method

To evaluate sensitivity of the AquAd method, we performed real-time PCR using a positive control plasmid containing the target sequence (a 156 bp amplicon) as a template. According to qualitative real-time PCR assay guidelines (Broeders et al., 2014; BVL, 2016a; ENGL, 2015), the templates were prepared by dilution to 6.25–1000 copies per reaction. > 25 copies per reaction was detected by mean Cq values ≦38.69 (Table 3). The control plasmid concentration at 12.5 copies per reaction was detected in 19 out of 21 reactions, indicating the lowest copy number that could be detected, at a 95% confidence rate ($LOD_{95\%}$), was 12.5–25 copies per reaction. Reactions with no positive control plasmid added had a 0% false positive rate.

To evaluate real-time PCR amplification efficiency, a standard curve (Cq value vs copy number) using the positive control plasmid at ≧25 copies (25, 50, 100, 1000 copies) per reaction was plotted. As shown in Fig. 3, the standard curve showed linearity (r^2 values 1.00) and the PCR amplification efficiency was calculated as 83% from the slope. These results indicated that linearity of the curve was sufficient, but the PCR amplification efficiency was slightly low compared with criteria (90–110%) for the quantitative real-time PCR methods suggested by Broeders et al. (2014) and ENGL (2015). As shown in Fig. S1, the linearity was not good for range from 6.25 to 25 copies per reaction, but much better for range from 25 to higher copy numbers per reaction. Hence, these results indicate that the AquAd detection method can detect quantitatively > 25 copies.

3.3. Reproducibility of the AquAd detection method

To evaluate reproducibility of the AquAd detection method, a collaborative trial involving three independent laboratories was performed. The genome size and a haploid genome mass of non-GM counterpart salmon have been estimated to be approximately 3.0 Gb and 3.27 pg, respectively (BVL, 2016a; Hardie & Hebert, 2003; Lien et al., 2016). Blind samples were prepared in such a way that each sample contained approximately 0, 7.5, 37.5 or 75 haploid genome equivalents, based on our $LOD_{95\%}$ results (Table 3). Consequently, the three laboratories received a set of randomized blind DNA samples consisting of four different concentrations of AquAd (0, 0.1, 0.5 and 1.0% [w/w]), replicated six times for each concentration. The results

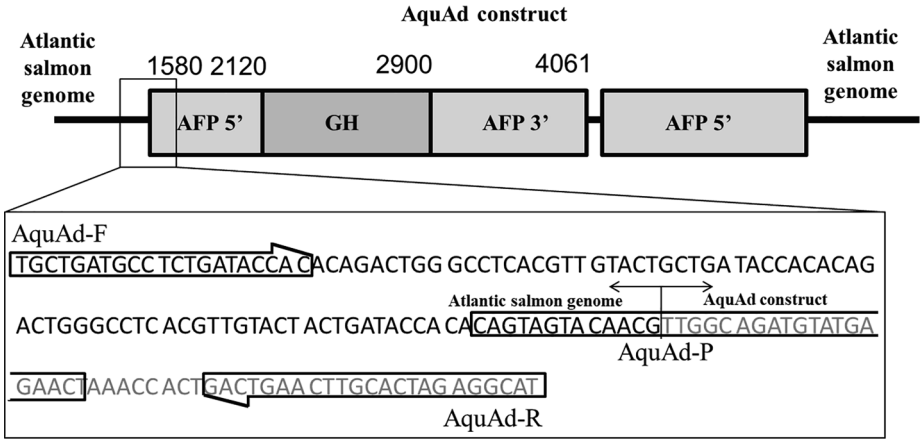

Fig. 1. Schematic diagram of the AquAd specific sequences. The AquAd-specific sequences (indicated within the arrows, described by Yaskowiak et al., 2006) that include the 5′ terminus-flanking region between the host Atlantic salmon genomic DNA and the transgenic DNA were targeted. AFP 5′, the 5′ promoter region derived from the antifreeze protein gene from Ocean pout; GH, the gene encoding a growth hormone from Chinook salmon; AFP 3′, the 3′ terminator region derived from the antifreeze protein gene from Ocean pout. Base pair numbers of the opAFP-GHc2 sequences were indicated according to the GenBank accession no. AY687640.1.

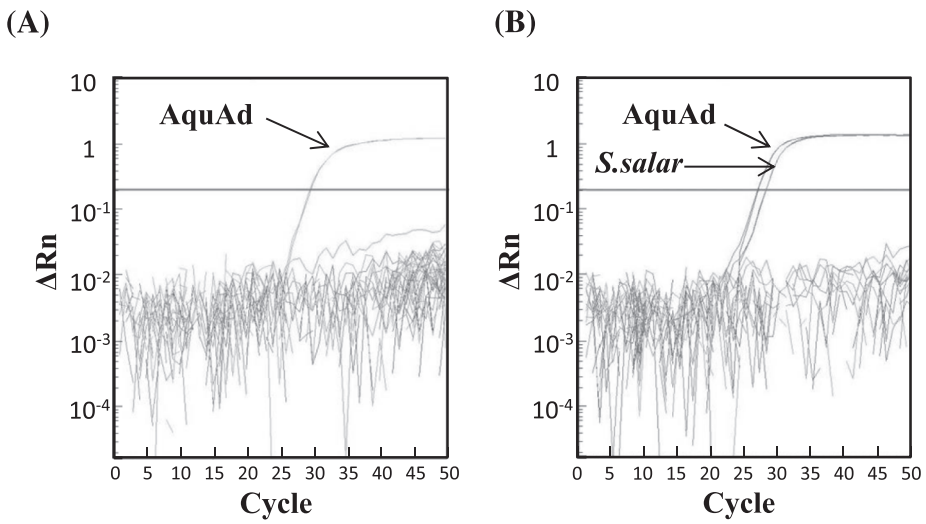

Fig. 2. Specificity test for AquAd and GH1 detection methods. The specificity test for AquAd detection method (A) and endogenous control gene GH1 detection method (B) were performed using 50 ng genomic DNA purified from AquAd and the other species listed in Table 2. The horizontal line indicated the threshold (ΔRn = 0.2).

from each laboratory showed that AquAd-specific DNA amplification (Cq value < 48) was observed in 0.1, 0.5 and 1.0% (w/w) AquAd samples, but not in any of the blank samples (0% [w/w] AquAd). Results were consistent among the three laboratories (Table S1). Cq values of some 0.1% (w/w) samples obtained using the AquAd method were relatively high (39.44 ± 1.48) and out of quantitative range at > 40 (Fig. S1), indicating that the AquAd method might not exhibit suitable accuracy at levels below 0.1% (w/w). The inter-laboratory testing showed no false results. Therefore, we consider that the AquAd detection method is reproducible to detect AquAd qualitatively if not quantitatively.

Table 3
LOD of AquAd detection method.

Copy number (/reaction)	Detection rate	Mean of Cq values	SD	RSD
1000	21/21	32.60	0.44	1.36
100	21/21	36.34	0.62	1.70
50	21/21	37.66	1.18	3.12
25	21/21	38.69	1.82	4.72
12.5	19/21	38.83	1.26	3.25
6.25	13/21	39.04	1.89	4.85
NTC	0/21			

NTC, no template control; SD, standard deviation; RSD, relative SD.

Table 2
Specificity test for the developed real-time PCR detection method.

Organism	Scientific name	NCBI taxonomy ID	AquAd detection method*		GH1 detection method*	
			test1	test2	test1	test2
Plants	*Allium cepa* L.	4679	–	–	–	–
	Apium graveolens Dulce Group	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	–	–	–	–
	Solanum lycopersicum	4081	–	–	–	–
	Solanum melongena L.	4111	–	–	–	–
	Solanum tuberosum L.	4113	–	–	–	–
	Triticum aestivum L.	4565	–	–	–	–
	Zea mays L.	4577	–	–	–	–
Animals	*Bos taurus*	9913	–	–	–	–
	Sus scrofa	9823	–	–	–	–
Fishes	*Hyperoglyphe japonica*	171196	–	–	–	–
	Oncorhynchus keta	8018	–	–	–	–
	Oncorhynchus kisutch	8019	–	–	–	–
	Oncorhynchus masou ishikawae	8021	–	–	–	–
	Oncorhynchus mykiss	8022	–	–	–	–
	Pagrus major	143350	–	–	–	–
	Scomber japonicus	13676	–	–	–	–
	Salmo salar (Atlantic salmon)	8030	–	–	27.89	27.90
	GM salmon (AquAd)	–	29.64	29.51	26.78	26.68
	No template control (NTC)	–	–	–	–	–

*Cq values were obtained from a duplicate test using 50 ng DNA as template at the threshold (ΔRn = 0.2).
-, no amplification plot obtained.

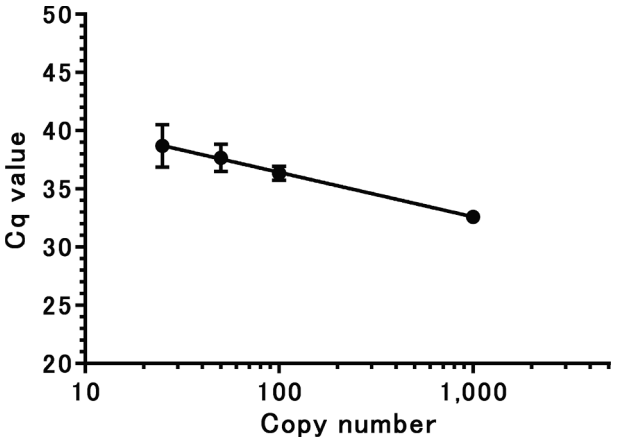

Fig. 3. A standard curve of the diluted positive control plasmids. The positive control plasmids at 25, 50, 100, 1000 copies per a real-time PCR test were used for the template. The standard curve obtained by 21-replicates was shown, and the coefficient of determination and amplification efficiency were 1.00 and 83%, respectively.

4. Discussion

Worldwide demand for fish is increasing and, therefore, increased aquaculture productivity is desirable. Approximately 50 species of fish have been subjected to genomic DNA modification (Cows et al., 2010). Most GM fish, including AquAd, have been developed for food production, because of the perceived advantages of aquaculture. It is possible that more GM fish will be developed and approved for food use in the near future. However, a screening strategy to confirm the presence or absence of GM fish is needed. Transgene construct-specific detection methods, which target two or more adjacent genetic elements in the transgene construct, have been reported. However, the transgenic-construct specific detection method does not distinguish between transgenic events if GM fish contain the same or similar transgenic constructs. To identify the presence of a particular GM fish, a specific detection method for the boundary sequence between the host genome and the transgene (i.e. event-specific method) is needed.

In the present study, we developed a real-time PCR detection method specifically for AquAd. According to international guidelines, the criterion for a reliable, qualitative, real-time PCR detection method is an LOD < 20–25 copies per reaction (Broeders et al., 2014; BVL, 2016a; ENGL, 2015). Results indicated that the $LOD_{95\%}$ of our AquAd method was slightly better (12.5–25 copies per reaction) than that specified in the criterion and still within the allowable range. Thus, our method would be useful to detect the presence of AquAd-derived ingredients in food products.

AquAd was the first GM animal approved for human food consumption and, therefore, great attention has been paid worldwide to regulatory approval. As is the case for GM crops, GM food regulations are unique among to counties and/ or regions, such as EU. Indeed, the labeling of GM products is mandatory in Japan at a threshold of 5% (Hino, 2002) and, in EU, at a threshold of 0.9% (Davison & Bertheau, 2008), whilst labeling regulations have not yet been enacted officially in some countries. Similar to GM crops, mandatory labeling regulation will also be implemented for GM animals in each country.

In this study, an inter-laboratory study demonstrated that the AquAd method was reproducible in detecting DNA in unprocessed fillets at a concentration of above 0.1% (w/w). Our AquAd method has an LOD < 0.9% and, therefore, given the lowest international threshold for labeling GM crops is 0.9% in EU, the method is compatible with the sensitivity required for AquAd regulation. Indeed, Japan has implemented this method officially since 2017 to detect AquAd for qualitative testing at quarantine stations and prefectural research institutes in Japan. Our detection methods would also be useful for monitoring labeling of foods containing AquAd, once authorized in Japan, just as in the case of GM papaya line 55–1, which is resistant to papaya ringspot virus infection, and went on to become the first commercialized GM fresh fruit for food use according to the Japanese regulations (Nakamura et al., 2013).

Previously, another group reported a specific detection method for an endogenous reference control gene (*GH1*) in Atlantic salmon (Hafsa et al., 2016). In this study, the test was performed using a real-time PCR primers and probe set, as described by Hafsa et al. (2016), but the amplification signal was extremely low (not over the signal threshold, data not shown). In the original paper, guanosine at the 5′ terminus of the real-time PCR probe was labeled with FAM. However, it is known that guanosine quenches fluorescence from some reporter dyes adjacent to it, meaning TaqMan probes with guanosine at the 5′ end are not recommended (Lie & Petropoulos, 1998; Malkki & Petersdorf, 2012). Accordingly, we re-designed the real-time PCR probe for GH1 (AM5PR-2, Table 1) by adding a thymidine to the 5′ terminus guanosine and labeling this with FAM. Subsequently, GH1 method fluorescence intensity improved, compared with the original real-time PCR probe (data not shown), and our results indicated that the GH1 method could detect Salmonidae, especially Atlantic salmon. To our knowledge, this method is the most specific for Atlantic salmon species among any other methods described so far (Debode et al., 2018; Hafsa et al., 2016). In this study, only Atlantic salmon in the genus *Salmo* were detected using the GH1 method, i.e. not the genus *Oncorhynchus*. The alignment of *GH1* sequences indicated that the region, within which the TaqMan probe (AM5PR) hybridizes, is more specific to Atlantic salmon than any primers and, thus, is critical to confer real-time PCR specificity for Atlantic salmon (Debode et al., 2018; Hafsa et al., 2016). Based on this evidence, a TaqMan assay using AM5PR-2 is recommended to discriminate Atlantic salmon from any other fish, including Salmonidae, over a SYBR green assay using primers only.

It has been reported that species names displayed on labels of some salmon products are not in agreement with the species in the products (Herrero, Vieites, & Espiñeira, 2011; Li, Li, Zhang, He, & Pan, 2013). This fraudulent labeling is a serious problem across the fish market sector. Thus, a regulatory system to monitor ingredients in products is becoming increasingly important. To confirm the presence of specific ingredients qualitatively, a detection method, without cross-reactivity with the other species, is indispensable. Thus, our AquAd detection method, in combination with the GH1 detection method, would be suitable to monitor for the presence of AquAd salmon in food products. Recently, we confirmed applicability of the GH1 detection method for detecting Atlantic salmon qualitatively in processed salmon commodities (Soga et al., 2019). However, we did not examine whether combining the AquAd and GH1 detection methods would be applicable to AquAd quantification in processed foods. Further examination is required to explore this.

In conclusion, we developed successfully an AquAd-specific detection method and improved an endogenous Atlantic salmon gene-specific detection method, using TaqMan real-time PCR. The methods are sensitive and specific to AquAd and were fully validated. Together, these method could be useful for detecting and/or monitoring AquAd ingredients in food products.

Declaration of Competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125426.

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