PCR Protocol

Method: QL-CON-GH-tFreeze-F/GH-tFreeze-R

Qualitative real-time PCR (TaqMan) method for detection of the junction between the growth hormone gene and the antifreeze terminator present in AquAdvantage® Salmon (Debode et al., 2017)

Target: Target is the junction region between the growth hormone gene (CS-GHc2) sequence from *Oncorhynchus tshawytscha* and the antifreeze terminator (T-AFP) from *Zoarces americanus*

Table 1 - Oligonucleotides

Name	DNA sequence of oligonucleotides	Final concentration in PCR
target: CS-GHc2-T-AFP		
GH-tFreeze-F	5'- ctc cac agg ttt tga cat gtt ca-3'	340 nM
GH-tFreeze-R	5'- gcc agc aag agc cca tct c -3'	340 nM
GH-tFreeze-P	5'-FAM- ttc cta atc tgt atc tgg gaa acc gaa ccc t -TAMRA-3'	540 nM

Amplicon length: 75 bp
Amplicon sequence: 5'-gccagcaagagcccatctccagggttcggtttcccagatacagattaggaactgaacatgtcaaaacctgtggag-3'

Table 2 – Addition of reagents

Total reaction volume	25 µl
sample DNA (up to 200 ng) or control DNA	5 µl
PCR buffer[1] (including $MgCl_2$, dNTPs, and DNA polymerase)	12.5 µl
primers GH-tFreeze-F and GH-tFreeze-R	see Table 1
probe GH-tFreeze-P	see Table 1
water	add to 25 µl
[1]TaqMan Universal Master Mix (No. 4324020)	

Table 3 – Temperature-time program

Step	Parameter		Temperature [°C]	Time [s]	Fluorescence measurement	Cycles
1	optional (for AmpErase®UNG incubation)		50	120	no	1
2	denaturation / polymerase activation		95	600	no	1
3	amplification	denaturation	95	15	no	50
		annealing/extension	60	60	yes	

Method performance:

Table 4 - Specificity

detection	experimentally verified (species/GMO)	theoretically verified (GenBank Acc.No.)
positive	DNA of AquAdvantage® salmon (obtained from AquaBounty Inc.)	AY594644
negative	*Salmonidae* family: Atlantic salmon Trout Rainbow trout Coho salmon Sockeye salmon Pink salmon Brook trout	./.

Limit of Detection (LOD): 10 copies/reaction (with plasmid DNA)

Reference material:
A control plasmid carrying the specific target sequence amplified by method QL-CON-GH-tFreeze-F/GH-tFreeze-R is available at:

Dr. Ir. Frédéric Debode
CRA-W
D4 Valorisation of agricultural products
Unit 16: Authentication and traceability of agricultural products
24, Chaussée de Namur
B-5030 Gembloux / Belgium
Phone: 00 32 (0) 81 62 03 51
Fax: 00 32 (0) 81 62 03 88
E-mail: f.debode@cra.wallonie.be

References:

Debode F, Janssen E, Marien A, Devlin RH, Lieske K, Mankertz J, Berben G (2017) Detection of transgenic Atlantic and Coho salmon by real-time PCR. Submitted for publication

GenBank Accession No. AY594644.1 (2004) Synthetic construct opAFP-GHc growth hormone I precursor gene, complete cds.

US patent 5545808A (1996) Transgenic salmonid fish expressing exogenous salmonid growth hormone.

by courtesy of Frédéric Debode Date: 10-10-2017