

The following supplement accompanies the article

Comparison of PCR methods for the detection of genetic variants of carp edema virus

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Diseases of Aquatic Organisms 126: 75–81 (2017)

Sequence alignment

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CEV_Genogroup_I      -----GTTTGTATGGAGT
OyamCEV              TGCAGGATCATTATCCTTGTATCTACAAGTACAATAGAAAGCAAGAAGTTTGTATGGAGT
CEV_Genogroup_Ia     -----GTTTGTATGGAGT
                      *****
                      CEFAS F          CEFAS Fn
CEV_Genogroup_I      ATCCAAAGTACTTAGATTAATAATTATCAATGAAATTTGTGTATTGTGTTTTGTTAGTCC
OyamCEV              ATCCAAAGTACTTAGATTAATGTTATCAATGAAATTTGTGTATTGTGTTTTGTTAGTCC
CEV_Genogroup_Ia     ATCCAAAGTACTTAGATTAATGTTATCAATGAAATTTGTGTATTGTGTTTTGTTAGTCC
                      *****
CEV_Genogroup_I      AAGAGTTTTCTTTCTCGTCATTTGTTACTTTTTGTAGTTGTTAATATTTGTGATAAGATT
OyamCEV              AAGAGTTTTCTTCTCATCGTTTGTACCTTTTGTAGTTGTTAATATTTGTGATAAGATT
CEV_Genogroup_Ia     AAGAGTTTTCTTCTCATCGTTTGTACTTTTTGTAGTTGCTTAATATTTGTGATAAGATT
                      *****
                      CEFAS_qF
CEV_Genogroup_I      TCCATTGGCATAAAAATCCTTCCCAGATTTGGGTTGAAACATGTTTTAGATTTTGTATAAT
OyamCEV              TCCATTAGCATAAAAATCCTTCCCAAATTTGTGTTGATACATGTTTTAGTGTTTTGTAGAT
CEV_Genogroup_Ia     TCCATTGGCATAAAAATCCTTCCCAAATTTGTGTTGAAACATGTTTTAGTGTTTTGTAGAT
                      *****
                      TiHo Sybr qF      CEFAS q Probe      CEFAS qR
CEV_Genogroup_I      TGTAGCATTTCCTAGTTTGTATGGCAAGAAACAAACTCTCTTACTGTAACCTCTGAGG
OyamCEV              TGTAGCATTTCCTAGTTTGTATGGCAAGAAACAAACTCTCTTACTGTAACCTCTGAGG
CEV_Genogroup_Ia     TGTAGCATTTCCTAGTTTGTATGGCAAGAAACAAACTCTCTTACTGTAACCTCTGAGG
                      *****
CEV_Genogroup_I      AATCTGATCTAGAATTCACAAATATGTAATCTCAAATTTGTTTGTGGAGTTTTTGAATA
OyamCEV              AATTTGATCTAGAATTCACAGAATGTAATCTCAAATTTGTTTGTAGAGTTTTTGAAGTA
CEV_Genogroup_Ia     AATTTGATCTAGAATTCACAGAATGTAATCTCAAATTTGTTTGTGGAGTTTTTGAAGTA
                      ***
CEV_Genogroup_I      TACTACTTCATCATAAATCCTAGAACTAGAGCAAGATTAGAAGTCATTGTCTTGTCAA
OyamCEV              TACTGTTTCATCACACAATCCTAGAACTAGAGCAAGATTAGAAGTCATTGTCTTATCGAA
CEV_Genogroup_Ia     TACTGTTTCATCATAAATCCTAGAACTAGAGCAAGATTAGAAGTCATTGTCTTATCGAA
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Primers

CEFAS end-point (Matras et al., 2017)

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CEFAS_F      ATGGAGTATCCAAAGTACTTAG
CEFAS_R      CTCTTCACTATTGTGACTTTG
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CEFAS nested end-point (Matras et al., 2017)

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CEFAS_nF     GTTATCAATGAAATTTGTGTATTG
CEFAS_nR     TAGCAAAGTACTACCTCATCC
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CEFAS probe (Matras et al., 2017)

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CEFAS_qF     AGTTTGTAKATTGTAGCATTTC
CEFAS_qR     GATTCCTCAAGGAGTTDCAGTAAA
CEFAS_q_Probe[FAM] AGAGTTTGTCTTCCATACAAACT [BHQ1]
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TiHo probe (Adamek et al., 2016)

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TiHo_qF      TTAGGAGGACAAGTAAAGTTACCA
TiHo_qR      GCAAGTTATTCGATGCCAACC
TiHo_q_Probe [FAM] CCAGCTCTACAAGGAAAGCAATTGA [BHQ1]
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Sequence alignment

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TiHo_Sybr_qR
CEV_Genogroup_I GACAGACATCTTATTCCAATCATCAATCTGGATTCTCTTCCAGAACATAAACATTTGCAAT
OyamCEV GACATTCATCTTATTCCAATCATCAATCTGAATTCCTTTCCAGAACATAAACATTTGCAAT
CEV_Genogroup_IIa GACAGACATCTTATTCCAATCATCAATCTGAATTCCTTTCCAGAACATAAACATTTGCAAT
****

CEV_Genogroup_I TTTAACTTGCTCTGGAATTGTATCAACGTATCCAATATCTTCTTTACTACGTAATTTGG
OyamCEV TTTAACTTGCTCTGGAATTGTATCAACGTATCCAATATCTTCTTTACTACGTAATTTGG
CEV_Genogroup_IIa TTTAACTTGCTCTGGAATTGTATCAACGTATCCAATATCTTCTTTACTACGTAATTTGG
*****
CEFAS Rn CEFAS R
ATGAGGTAGTACTTTGCTA CAAAGTCACAATAATGAAGAGTTGTCATTTTAATTTGTTG
OyamCEV ATGAGGTAGTACTTTGCTA CAAAGTCACAATAATGAAGAGTTGTCATTTTAATTTGTTG
CEV_Genogroup_IIa ATGAGGTAGTACTTTGCTA CAAAGTCACAATAATGAAGAGTTGTCATTTTAATTTGTTG
*****

CEV_Genogroup_I TAGATCAATTTCTGCAAATTTGATGTATATCAGGAATATCAAATTTAACCATATTTGCAA
OyamCEV TAGTCCAATTTCTGCAAATTTGATGTATATCAGGAATATCAAATTTAACCATATTTGCAA
CEV_Genogroup_IIa TAGTCCAATTTCTGCAAATTTGATGTATATCAGGAATATCAAATTTAACCATATTTGCAA
***

CEV_Genogroup_I TGGATTTGCTCCTGCTGCGGCGCCATTACATAATTAGAATCACGAAGTTCAGGATCTCT
OyamCEV TGGATTTGCTCCTGCTGCTGGT---GCTGCCATTACGTAATTAGAATCGCGAAGTTCAGGATCTCT
CEV_Genogroup_IIa TGGATTTGCTCCTGCTGCTGGT---GCTGCCATTACGTAATTAGAATCACGAAGTTCAGGATCTCT
*****
Oyamatsu_F
CEV_Genogroup_I TACTGCTGATGTTGCATTTTCTTTGAGAATGAACGGAATCAACAAGTTGATATGCATTTGC
OyamCEV TGCTGCTGCTGTTGCAACCATTTGAGAAATGAACCGAATCAACAAGTTGATATGCATTTGC
CEV_Genogroup_IIa TGCTGCTGCTGTTGCAACCATTTGAGAATGAACCGAATCAACAAGTTGATATGCATTTGC
*

CEV_Genogroup_I ATTTGCATCAAAAGTAACTTACGCTGGGAATGATTGAGACAAAGTAGAATTTCTTGA
OyamCEV ATTTGCATCAAAAGTAACTTACGCTGGGAATGATTGAGACAAAGTAGAATTTCTTGT
CEV_Genogroup_IIa ATTTGCATCAAAAGTAACTTACGCTGGGAATGATTGAGACAAAGTAGAATTTCTTGT
*****

CEV_Genogroup_I GTAATGCATATCTTGAGAACCAGCTACTCCACCTTGGTTAACTCCAAGAGCATAATGATA
OyamCEV ATAATGTATATCTTGAGAACCAGCTACTCCACCTTGGTTAACTCCAAGAGCATAATGATA
CEV_Genogroup_IIa GTAATGCATATCTTGAGAACCAGCTACTCCACCTTGGTTAACTCCAAGAGCATAATGATA
*****

CEV_Genogroup_I TTCAAGATCTAGTCTAATTTGGTCTGGAGAATAAGTGTATGATTTAATTCATATAGTTT
OyamCEV TTCAAGATCTAGTCTAATTTGGTCTGGAGAATAAGTGTATGATTTAATTCATATAGTTT
CEV_Genogroup_IIa TTCAAGATCTAGTCTAATTTGGTCTGGAGAATAAGTGTATGATTTAATTCATATAGTTT
*****

CEV_Genogroup_I AATGAAGTCTCATAATTACTTTGTCCAAATAAAGTTAGATAAAATAGTTTTAGGATTGA
OyamCEV AATGAAATGCTCATAATTACTTTGTCCAAATAAAGTTAGATAAAATAGTTTTAGGATTGA
CEV_Genogroup_IIa AATGAAATGCTCATAATTACTTTGTCCAAATAAAGTTAGATAAAATAGTTTTAGGATTGA
*****
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Primers

TiHo SYBRGreen (Adamek et al., 2017)

TiHo_Sybr_qF CATTTCCTAGTTTGTATGGCAAG
TiHo_Sybr_qR TGATGATTGGAATAAGATGTCTGTC

Oyamatsu end-point (Oyamatsu et al., 1997)

Oyamatsu_F GCTGTTGCAACCATTTGAGA
Oyamatsu_R TGCAAGTTGCTCCTAATCCT

Oyamatsu nested end-point (Oyamatsu et al., 1997)

Oyamatsu_nF GCTGCTGCACTTTTAGGAGG
Oyamatsu_nR TGCAAGTTATTTGATGCCA

Sequence alignment

Primers

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                Oyamatsu Fn      TiHo qF      TiHo q_Probe
CEV_Genogroup_I  AGCAAGAGCTCCTGCGCTTTTAGGAGGACAAGTAAAATTGCCATCAGTT-CCAACAAGGA
OyamCEV          AGCAAGAGCTGCTGCACTTTTAGGAGGACAAGTAAAGTTACCACCAGCTCCTACAAGGA
CEV_Genogroup_IIa AGCAAGAGCTGCTGCACTTTTAGGAGGACAAGTAAAGTTACCACCAGCT-CCTACAAGGA
*****  ****  *****

CEV_Genogroup_I  AAGCAATTGATTTTACTTGAAGAACATTCTAGAAGAGAAGAGAAATTCTCAAGAATTA
OyamCEV          AAGCAATTGAATTTTACTTGAAGAACAATCTAGAAGATTGGAGAAATTCTCAAGAATTA
CEV_Genogroup_IIa AAGCAATTGATTTTACTTGAAGAACATTCAGAAGATTAGAGAAATTCTCAAGAATTA
*****  ****  *****

                TiHo qR
CEV_Genogroup_I  GAATTGCAACTTCTAGTCTTCCAAATCCTCTAGATTTAGATTTAGATTGGCATGTAAAT
OyamCEV          GAATTGCAACTTCTAGTCTCTAGTTTTTCTAGATTTAGATTTAGGTGGCATCGAAAT
CEV_Genogroup_IIa GAATTGCAACTTCTAGTCTCTAGTTTTTCTAGATTTAGATTTAGGTTGGCATCGAAAT
*****  ****  *****

Oyamatsu_Rn
CEV_Genogroup_I  AACTTGCA-----
OyamCEV          AACTTGCATAATCTAGAAGTTCAATCAACATCAAATGTACTTACATCAAATAGGAAAGGAT
CEV_Genogroup_IIa AACTTGCA-----
*****

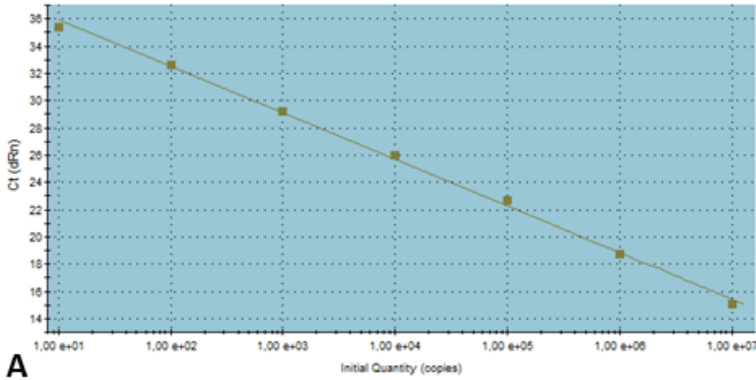
Oyamatsu_R
CEV_Genogroup_I  -----
OyamCEV          TAGGAGCAACCTGCA
CEV_Genogroup_IIa -----

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Figure S1. DNA sequence alignment (performed with Clustal Omega) presenting sequence differences in a fragment of the P4a core protein encoding DNA obtained from the genogroup I and IIa of CEV compared with the initial CEV sequence obtained by Oyamatsu (1996). Highlighted are the positions and sequences of PCR primers and probes used in this work. Sequence indicated by underlined italics was used for phylogenetic studies and the selection of samples for the comparison of PCR methods.

TiHo SYBRGreen qPCR 10¹ to 10⁷ Standard Curve

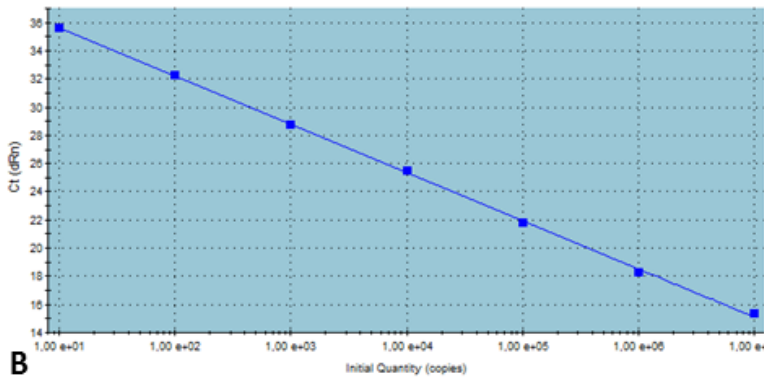
Log fit values
■ SYBR Standards, RSq 0.998
— SYBR, Y = -3.409*LOG(X) + 39.29, Eff. = 96.5%



A

TiHo probe qPCR 10¹ to 10⁷ Standard Curve

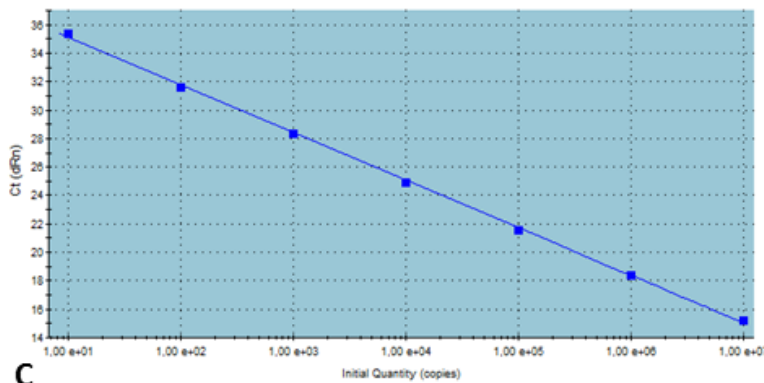
Log fit values
■ FAM Standards, RSq 0.999
— FAM, Y = -3.419*LOG(X) + 39.05, Eff. = 96.1%



B

CEFAS probe qPCR 10¹ to 10⁷ Standard Curve

Log fit values
■ FAM Standards, RSq 0.999
— FAM, Y = -3.342*LOG(X) + 38.42, Eff. = 99.2%



C

Figure S2: Standard curves based on supercoiled plasmid standards ranging from 10¹ to 10⁷ copies of CEF *p4a* gene. (A) standard curve for TiHo SYBRGreen qPCR, (B) standard curve for TiHo probe qPCR, (C) standard curve for CEFAS probe qPCR.

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