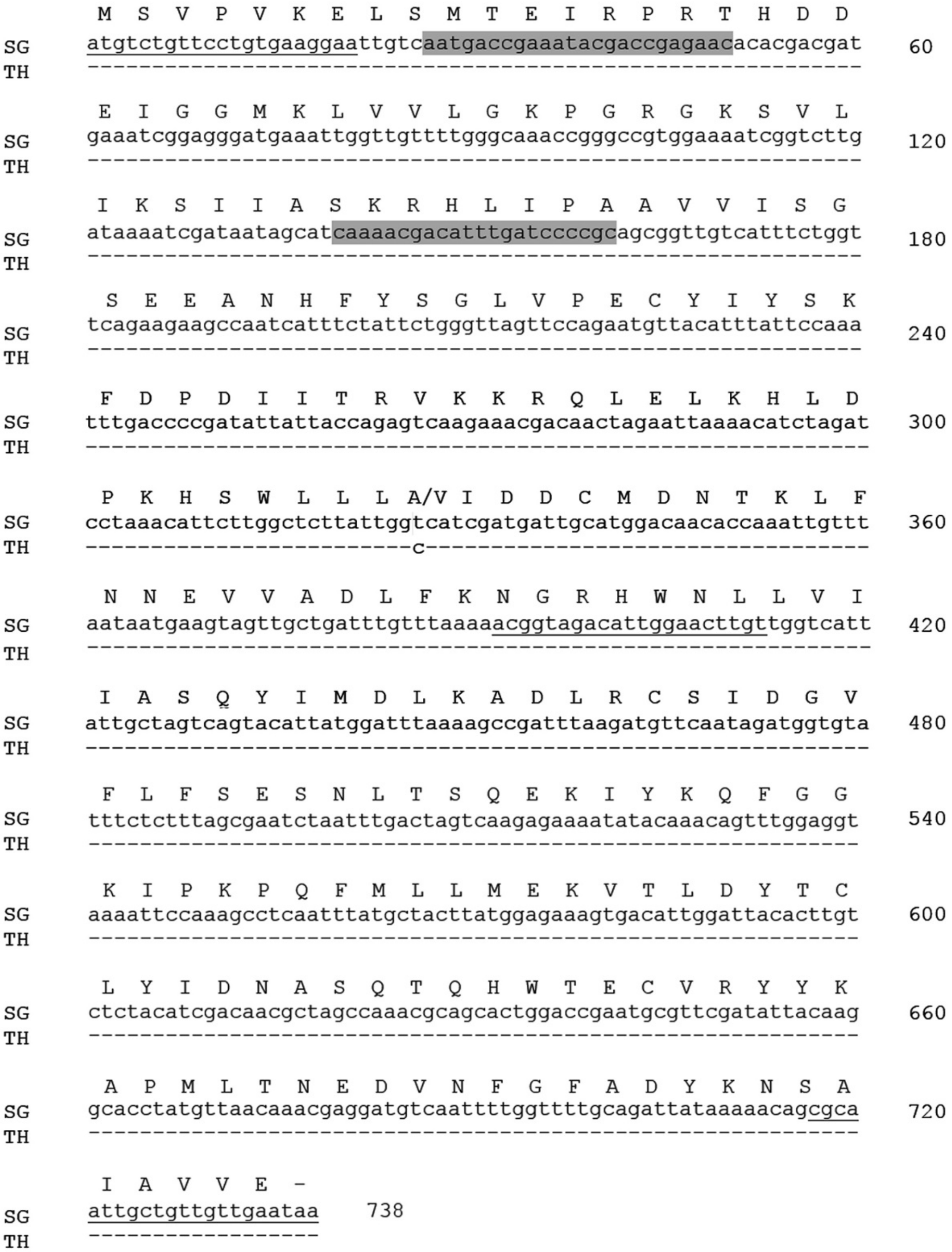

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        M  S  V  P  V  K  E  L  S  M  T  E  I  R  P  R  T  H  D  D
SG     atgtctgttcctgtgaaggaattgtcaatgaccgaaatacgaccgagaacacacgacgat    60
TH     ------------------------------------------------------------

        E  I  G  G  M  K  L  V  V  L  G  K  P  G  R  G  K  S  V  L
SG     gaaatcggagggatgaaattggttgttttgggcaaaccgggccgtggaaaatcggtcttg    120
TH     ------------------------------------------------------------

        I  K  S  I  I  A  S  K  R  H  L  I  P  A  A  V  V  I  S  G
SG     ataaaatcgataatagcatcaaaacgacatttgatccccgcagcggttgtcatttctggt    180
TH     ------------------------------------------------------------

        S  E  E  A  N  H  F  Y  S  G  L  V  P  E  C  Y  I  Y  S  K
SG     tcagaagaagccaatcatttctattctgggttagttccagaatgttacatttattccaaa    240
TH     ------------------------------------------------------------

        F  D  P  D  I  I  T  R  V  K  K  R  Q  L  E  L  K  H  L  D
SG     tttgaccccgatattattaccagagtcaagaaacgacaactagaattaaaacatctagat    300
TH     ------------------------------------------------------------

        P  K  H  S  W  L  L  L A/V I  D  D  C  M  D  N  T  K  L  F
SG     cctaaacattcttggctcttattggtcatcgatgattgcatggacaacaccaaattgttt    360
TH     -------------------------c----------------------------------

        N  N  E  V  V  A  D  L  F  K  N  G  R  H  W  N  L  L  V  I
SG     aataatgaagtagttgctgatttgtttaaaaacggtagacattggaacttgttggtcatt    420
TH     ------------------------------------------------------------

        I  A  S  Q  Y  I  M  D  L  K  A  D  L  R  C  S  I  D  G  V
SG     attgctagtcagtacattatggatttaaaagccgatttaagatgttcaatagatggtgta    480
TH     ------------------------------------------------------------

        F  L  F  S  E  S  N  L  T  S  Q  E  K  I  Y  K  Q  F  G  G
SG     tttctctttagcgaatctaatttgactagtcaagagaaaatatacaaacagtttggaggt    540
TH     ------------------------------------------------------------

        K  I  P  K  P  Q  F  M  L  L  M  E  K  V  T  L  D  Y  T  C
SG     aaaattccaaagcctcaatttatgctacttatggagaaagtgacattggattacacttgt    600
TH     ------------------------------------------------------------

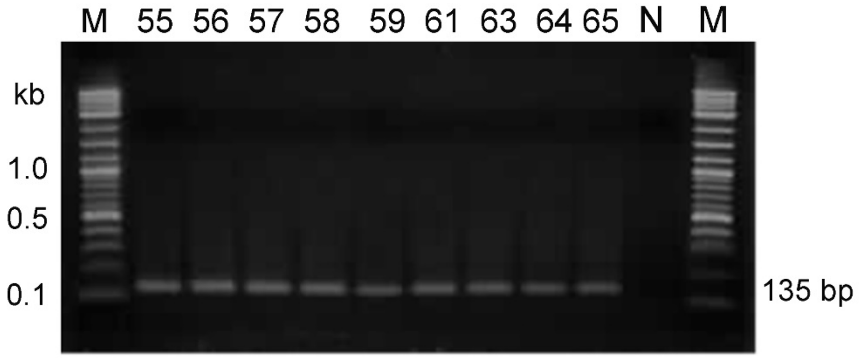
        L  Y  I  D  N  A  S  Q  T  Q  H  W  T  E  C  V  R  Y  Y  K
SG     ctctacatcgacaacgctagccaaacgcagcactggaccgaatgcgttcgatattacaag    660
TH     ------------------------------------------------------------

        A  P  M  L  T  N  E  D  V  N  F  G  F  A  D  Y  K  N  S  A
SG     gcacctatgttaacaaacgaggatgtcaattttggttttgcagattataaaaacagcgca    720
TH     ------------------------------------------------------------

        I  A  V  V  E  –
SG     attgctgttgttgaataa    738
TH     ------------------
```

Figure S1. Aligned *ATPase* gene sequences of SDDV strains from Thailand (TH, MH152407) and Singapore (SG, KR139659; ORF_035L) showing regions targeted by primers used in the SYBR-Green qPCR assay described here (shaded) and in the semi-nested PCR described in Charoenwai et al. (2019) (underlined).

a) Gradient PCR



b) Melt curve of qPCR

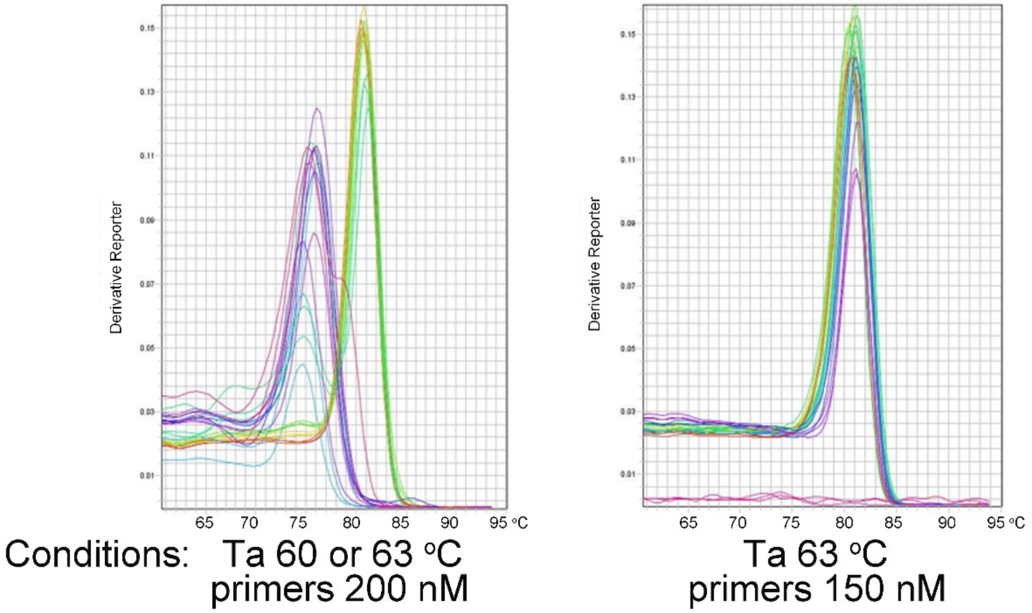


Figure S2. Optimizing the SYBR Green SDDV qPCR conditions. (a) Conventional gradient PCR over a 55-65°C Ta range using the qSDDV-AF/AR primer pair and SDDV-infected fish DNA (N = no template control), (b) Melt curve analysis of products of qPCR assays using a Ta of either 60 or 63°C and 200 nM each primer showing 2 peaks (left) and assays using a 63°C Ta and 150 nM each primer showing a single specific peak (right).

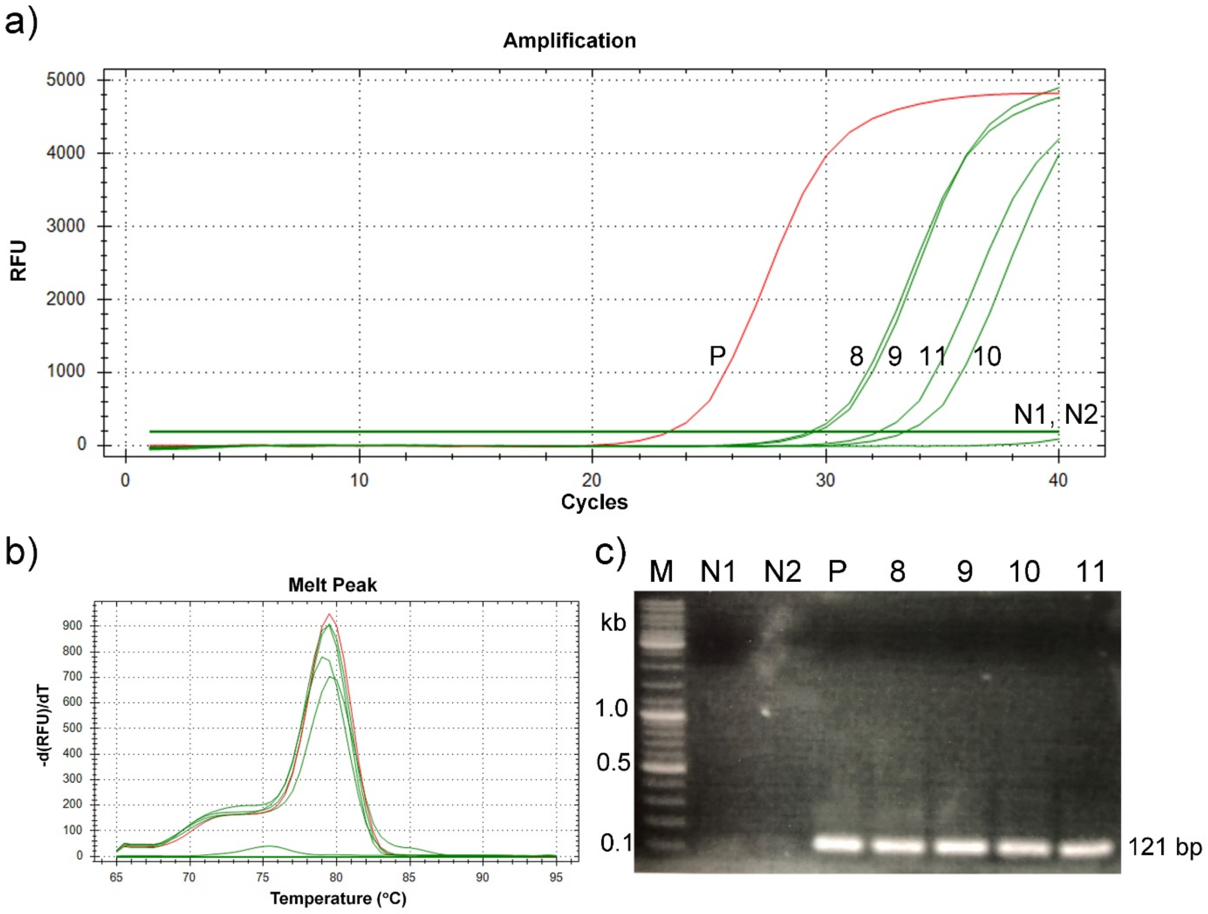


Figure S3. Data from an alternative SDDV SYBR Green qPCR assay using primers targeting the major capsid protein (*MCP*) gene to confirm positives among overtly-healthy fish DNA samples detected using the *ATPase* gene-specific qPCR. Data are shown for representative fish from Farms 8 to 11. P = SDDV positive fish DNA, N1 = no template control, N2 = SDDV-free fish sample. (a) *MCP* gene qPCR amplification plot, (b) melt peak analysis and (c) amplicon (121 bp) detected by electrophoresis in a 1.5% agarose gel.