**ADDITIONAL FILE**

**SUMMARY**

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# **1. Supplementary methods**

# **1.1. Plus/Minus assay**

The real-time PCR-based reaction "Plus/Minus" was performed for a final volume of 25 μl, containing Power SYBR® Green Mastermix (Applied Biosystems, USA); Primer forward 10mM; Reverse primer 10mM and 2μl DNA sample, complete with nuclease-free water. PCR amplification was performed in a real-time thermocycler ABI 7300 (Applied Biosystems, USA). The initial mixture was heated to 95 ° C for 10 minutes, followed by 40 cycles, each cycle consisting of two steps, first denaturation at 95° C for 15 seconds, hybridization and polymerization at 62° C for 1 minute. Fluorescence reading was performed at the end of the polymerization phase of each cycle. Positive (DNA extracted from an *Escherichia coli*) and negative (water) controls were included. A final dissociation step was performed to determine the fusion temperature (Tm) of amplicon in each case.

# **1.2. Real-time PCR assay**

To create the standard samples necessary for absolute quantification in qPCR, a plasmid was synthesized containing the DNA fragment of *Lawsonia intracellularis*, to be amplified. The fragment was inserted into the pGEM®-T Easy plasmid using the pGEM®-T Easy vector System kit (Promega, USA). This plasmid was cloned into highly competent JM109 cells and subsequently extracted with the QIAprep Spin Miniprep kit (Qiagen, USA). Finally, the concentration of the extracted plasmid was quantified by measuring DNA absorbance with the Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., USA).

After knowing the concentration of the extracted plasmid, the number of copies of it was calculated to subsequently make serial dilutions with a known number of copies of the plasmid per microliter (μl). Specifically, decimal dilutions were used from 107 copies/μl, up to 101 copies/μl.

The real-time PCR reaction was performed for a final volume of 25 μl, containing Power SYBR® Green Mastermix (Applied Biosystems, USA); Primer forward 10mM; Reverse primer 10mM and 2μl DNA sample, complete with nuclease-free water. PCR amplification was performed in a real-time thermocycler ABI 7300 (Applied Biosystems, USA). The initial mixture was heated to 95° C for 10 minutes, followed by 40 cycles, each cycle consisting of two steps, first denaturation at 95° C for 15 seconds, hybridization and polymerization at 62° C for 1 minute. Fluorescence reading was performed at the end of the polymerization phase of each cycle. Positive (DNA extracted from an E. coli) and negative (water) controls were included. A final dissociation step was performed to determine the fusion temperature (Tm) of amplicon in each case.