Primer Validation Protocol

1. Prepare a Serial dilution of the genomic DNA template by first adding 950μl molecular grade water to a 1.7ml microfuge tube. Add 50μl of genomic DNA* (0.1μg/μl) to water and vortex. Label this tube #1.

2. Add 800μl of molecular grade water to 4 more 1.7ml microfuge tubes and label them #2 - 5. Add 200μl of tube #1 to tube #2 and vortex. Repeat this step for dilutions tubes #3, #4, #5 (ie. 200μl from tube #2 into tube #3, etc).

3. Pipette 5μl of genomic DNA from tube #1 into first 2 wells, 5μl of tube #2 into next 2 wells, etc. until all 5 dilutions are in the first 10 wells. In the final 2 wells pipette 5μl of Molecular Grade water to use as a control to check for primer-dimer formation.

4. Prepare a 2:1 mixture of Biorad SYBR Green supermix (Cat. #170-1882) and primer pair stock mixture (500 nM, each primer). This can be done for 8 different sets of primer pairs on a single 96 well plate (one per row). Pipette 15μl of Supermix/Primer Pair mix into each well on a 96 well plate for a total of 20μl for the reaction (Final primer concentration is 125nM).

5. Obtain clear optical tape and without touching the edges, place tape onto top of the plate and press down using a plate sealer. Tear sides off of tape.

6. Centrifuge PCR plate for 2 minutes at ~2500rpm to get all of the liquid to the bottom of the wells.

7. RT-PCR Protocol Setup (Bio-Rad myIQ or iCycler):
   1. Begin with a 3 min step at 95°C
   2. 40 cycles with a step of 10 sec at 95°C, 45 sec at 65°C, and 20 sec at 78°C (Data acquisition step).
   3. One step of 1 min at 95°C.
   4. One step of 1 min at 55°C
   5. 80 cycles of 10 sec each starting at 55°C with a 0.5°C increment at each step up to 95°C (Melting point step to check for primer-dimer formation).

8. Plate Setup:
   1. Label duplicate wells as 1, 2, 3, 4, 5 and (−) for water control.
   2. Change standard quantity to 1 for all wells.

*Use genomic DNA for the organism that the primers are designed for.