1. Prepare a master mix of all the components listed below, except the (c)DNA template. Make sufficient mix for the total number of samples, plus one positive control, two water controls, and one spare reaction to allow for pipeting errors (*see* **Note 13**).

   For 1 × 50-µL reaction: 37.8 µL sterile ddH_2O, 1.0 µL dNTPs (10 m*M*) (*see* **Note 14**), 5.0 µL PCR buffer (10X), 3.0 µL $MgCl_2$ (25 m*M*) (*see* **Note 15**), 0.5 µL forward primer (50 µ*M*), 0.5 µL reverse primer (50 µ*M*), 0.2 µL *Taq* polymerase (5 U/µL) (*see* **Note 16**), 2.0 µL cDNA template.
2. Put 48 µL of mix into PCR tubes, and add 2 µL of DNA template. If required, add sufficient mineral oil (1 drop/50 µL) to cover each reaction.
3. PCR-amplify by placing in thermocycler, programmed as follows (*see* **Note 17**):
   a. 1 cycle: 95ºC for 2–3 min (initial denaturation).
   b. 30–45 cycles: 94°C for 10–60 s (denaturation), 45–70°C for 30 s (annealing), 72–74°C for 30–60 s (extension).
   c. 1 cycle: 72–74 C for 5 min (final extension).
4. Store samples in refrigerator or freezer till ready for electrophoresis.

*3.4.1. Detection of PCR Products by Gel Electrophoresis (*see ***Note 18****)*

1. Dissolve 1–2% (w/v) agarose in 0.5X TBE buffer by heating, and cool to ~50–60°C before pouring gel on level surface.
2. Once set, load 5–15 µL of PCR reaction plus 1–3 µL 6X loading dye into each well, and electrophorese samples at 4–10 V/cm distance between electrodes for desired length of time.
3. Visualize DNA bands on UV transilluminator after staining for 30 min in dilute ethidium bromide solution (0.5 µg/mL) (*see* **Note 19**).

3.5. Quantification of Product

PCR will be exponential under optimum primer, template, and reaction buffer conditions. However, because of the presence and generation of polymerase inhibitors, suboptimal cycling parameters, degradation in polymerase activity, and unsuitable template:primer ratios, the amplification efficiency is often not exponential, particularly after 15–20 PCR cycles. Thus, to discriminate between true and false negatives, and for quantification purposes, the reaction requires the inclusion of a known amount of amplifiable template in each tube as an internal control from which the amplification efficiency can be determined.

The internal control template should be amplified at the same rate as the target, because small differences in amplification will result in large differences in product yields. However, if its sequence is too similar, heteroduplexes can form in later cycles and interfere with amplification. The most important factor controlling the amplification rate is usually the primer sequences *(7)*, if G/C content and the presence of secondary structure are similar for control and