target templates ***(9)***. The use of the same primer annealing sequences in target and control template is recommended, therefore, and is termed competitive PCR. To quantify the target template, known molar amounts of control template are added into series of reaction tubes containing a set volume of the target template. Target and control products must be distinguishable by size (if necessary, after restriction digestion) or specific hybridization. The amount of control template giving the same yield of product represents the amount of target template.

The advantage of using competitive PCR is that quantification is possible even after less than exponential amplification. If the amplification efficiencies are the same for target and control template throughout the PCR cycles, then the amount of initial template can be calculated by comparison with the yield from a known amount of control template, and use of the equation $X = I(1 + E)^n$, where X = final quantity of product, I = amount of starting template, E = amplification efficiency (maximum value of 1), n = number of cycles (*see* **ref. *10***).

3.5.1. Construction of an Internal Control

Considerable care must be taken to find an internal control that is amplified at the same rate as the target, but does not hinder exponential amplification by allowing heteroduplex formation. Moreover, for RNA viruses, because reverse transcription (RT) is rarely 100% efficient and RNases may degrade some of the target during RNA extraction, there is a need for an internal RNA control, which is added to the sample before or during extraction.

3.5.2. Construction of Control DNA Template

1. Choose a control template for amplification of similar G/C content and size (within 200 bp) as the target, yet easily differentiated by gel electrophoresis.
2. Carry out 30 specific amplification cycles of the chosen control sequence with primers for that template that have been modified by addition of the target template primer sequences at their 5' ends.
3. Precipitate PCR product by adding 0.1 vol 3*M* sodium acetate, pH 5.5, and 2.5 vol 95% ethanol. Incubate at –70ºC for >30 min, centrifuge (12,000*g*, 15 min), wash pellet in 70% ethanol, dry, and resuspend in 50 µL sterile ddH_2O.
4. Reamplify 2 µL of precipitated template with the target template primers and precipitate, wash, and resuspend, as in **step 3**. Run 3 µL of product on an agarose gel to check for single product of desired size, and quantify concentration by UV spectroscopy (260 nm) or a fluorimeter (e.g., Hoechst model TKO 100).
5. Check whether the amplification efficiency of the control template equals that of the target template. Add equal copy numbers of control and target template to replicate 50 µL PCR reaction mixes, and remove 5-µL samples of the reaction mixes after 15, 20, 25, 30, and 35 cycles. Electrophorese on an agarose gel and