8. The use of oligo(dT) primers for RT of polyadenylated viruses generally only gives rise to 2–3 kb cDNAs. Random hexamers will generate cDNA from all RNA, and so may be preferrable.
9. Avian myeloblastosis virus (AMV) has a higher RNase H activity than Moloney murine leukemia virus (MMLV), and hence is less suitable for generation of long cDNAs. The best enzyme for templates in which secondary structure interferes with transcription would be the thermostable polymerase *rTth* (Perkin-Elmer). The latter can be used both for RT in presence of $MnCl_2$, and for PCR in presence of $MgCl_2$, after chelating out the manganese ion with EGTA. Addition of EGTA must be very accurate to avoid different $MgCl_2$ concentrations in different tubes.
10. It is important for the detection of double-stranded (ds) RNA to work quickly to avoid renaturation of strands.
11. RT and PCR can be performed in the same mix ***(2)***, but for difficult or low template numbers it is preferable to carry out RT and PCR separately under optimal reaction conditions for each enzyme.
12. Kwok and Higuchi ***(22)*** suggested numerous ways to avoid false positives in PCR, such as the use of positive-displacement pipets, separate work areas for handling of PCR products, regular changing of gloves, and the use of uracil primers followed by uracil DNA-glycosylase (UDG) treatment before the first PCR cycle. A simple precaution is to treat all tips, tubes and solutions with 10 min of UV, using, e.g., the Amplirad UV irradiation chamber (Genetic Research Instrumentation, Dunmow, Essex, UK).
13. PCR conditions given are meant as a starting point, from which reaction conditions for each different PCR test should be optimized. Specificity of primer annealing is primarily affected by temperature, magnesium ion concentration, and the concentration of primers and DNA polymerase. Annealing temperatures are generally between 55 and 72ºC, and a good starting point for testing new primers is 5–10ºC below the lowest melting temperature (T_m) of the primers used. Improved specificity of the primers can be achieved by increasing temperature, but care should be taken to avoid temperatures that reduce the amplification efficiency of the target. Note that dNTPs also chelate magnesium ions, so dramatic changes in their concentration will have a concomitant effect on the free magnesium concentration. One of the commonest reasons for a usually successful PCR to fail is too low a concentration of free magnesium, because of contaminating chelators, e.g., EDTA from extraction buffers, or increased concentrations of dNTPs.
14. The concentration of dNTPs should be 20–200 μ*M* for optimal balance between fidelity, specificity, and yield; a concentration above 200 μ*M* will result in increasingly poor fidelity. Primer annealing is aided by 50 m*M* KCl, but higher concentrations, or NaCl at 50 m*M*, will inhibit *Taq* activity. Commercially supplied PCR buffers usually contain 10–50 m*M* Tris-HCl, pH 8.0–9.0, but this may not be optimum for some reactions, e.g., long-distance PCR works more effectively at 25 m*M* Tris, pH 9.0. Nonionic detergents (0.5% Tween-20, or Triton X-100), gelatin, or BSA are often included to help stabilize the enzyme.