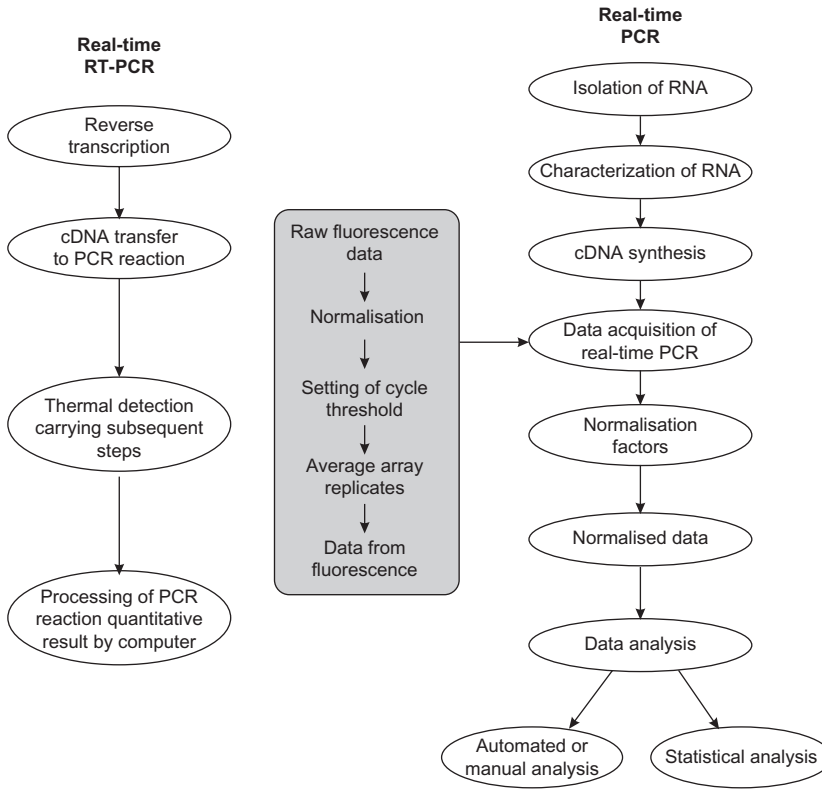

FIG. 6.6 Steps involved in real-time RT-PCR and real-time PCR.

attributed to its variable quality (Adams and Kelly, 1994; Bieche et al., 1999; Chiocchia and Smith, 1997; Bustin and Dorudi, 1998; Ståhlberg et al., 2005; Freeman et al., 1996; Ferre, 1992). The template abundance determines the efficiency of RNA to cDNA conversion. The nucleic acid present in RT-reaction may also affect the efficiency of RNA to cDNA conversion. In addition to this, different priming approaches also describe the efficiency of conversion. Commonly used approaches include random hexamers, specific primers, and oligo-dT primers. Oligo-dT and random primers maximize the mRNA molecules, which can be analyzed using a small RNA sample, whereas background priming is decreased by the mRNA-specific primers. When random-primers are used, the RT is primed at multiple origins, thereby producing more than one cDNA target per original mRNA target. Moreover, rRNA is involved in the cDNA synthesis, which can cause problems due to the low level of mRNA target of interest. This may lead to wrong quantization and disproportionate priming. Zhang and Byrne (1999) demonstrated how random hexamers overestimated mRNA copy numbers up