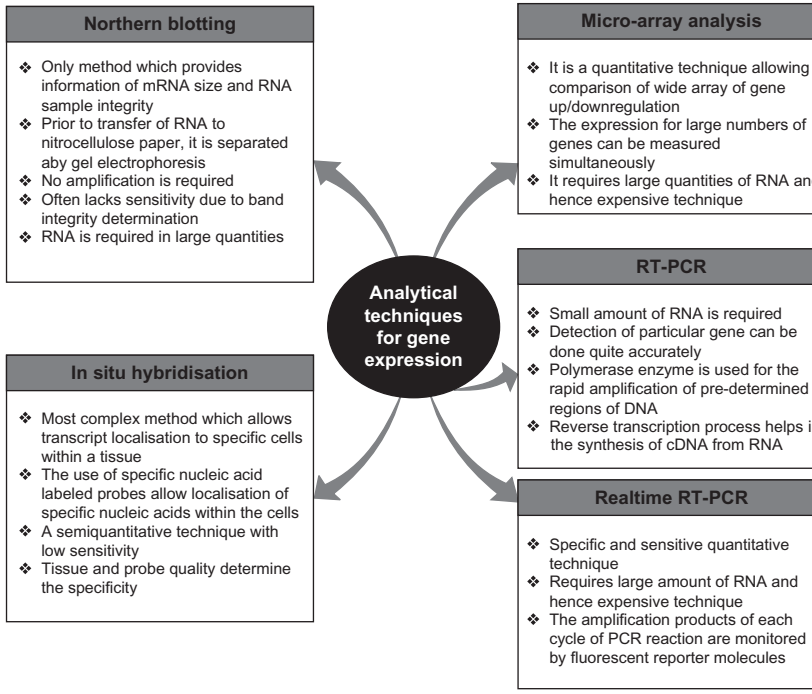

FIG. 6.5 Different techniques for gene expression (Jell and Stevens, 2006; Ståhlberg et al., 2005; Freeman et al., 1996; Ferre, 1992; Zhang and Byrne, 1999).

6.5 REAL-TIME PCR AS A PRIMARY TOOL FOR GENE EXPRESSION

Real-time PCR allows the analysis of different samples from as little as one cell for the same experiment. This technique can compare mRNA levels for different sample populations, along with characterizing mRNAs expression and analyzing RNA structure. RT-PCR is a complex technique, with interdependence of various parameters. Hence, the primary aim of assay, reaction fidelity, sensitivity, and specificity must be clearly investigated. Fig. 6.6 demonstrates the steps involved for measuring gene-expression using real-time PCR. Before proceeding, two things must be well understood, that is, reverse transcription and polymerase chain reaction, which are discussed in detail in following sections.

6.5.1 Reverse Transcription

For the RT-PCR array, RNA cannot act as a template for the PCR; hence, it is necessary to convert the RNA template into complimentary DNA (cDNA). To avoid the lack of reproducibility observed in RT-PCR, conversion of RNA to cDNA is an imperative step. Purified RNA can be rather unstable,