mRNA Quantitation Techniques: Considerations for Experimental Design and Application

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Studies of the regulation of gene expression rely upon techniques for the identification and quantitation of mRNA species coding for specific proteins. Several methods have been developed for this purpose, each offering distinct advantages and disadvantages. Rather than provide a comprehensive review of the current mRNA quantitation techniques, it is the intention of this article to discuss three of the most widely used techniques with particular attention to applications and limitations of each. The three techniques that will be discussed are Northern blot analysis, ribonuclease protection assay and quantitative reverse transcriptase-coupled polymerase chain reaction (RT-PCR).

Northern blot analysis

Northern blotting is the RNA counterpart of the Southern blot technique that was developed for DNA analysis by E. M. Southern in 1975 (Southern 1975). This method involves fractionating RNA species on the basis of size by denaturing gel electrophoresis followed by transfer of the RNA onto a membrane by capillary, vacuum or pressure blotting (Sambrook et al. 1989). The RNA is then permanently bound to the membrane in an apparent noncovalent interaction via exposure to short wave ultraviolet light or by heating at 80°C in a vacuum oven. RNA sequences of interest are detected on the blot by hybridization to a specific labeled probe. Probes for Northern blot detection generally contain full or partial cDNA sequences and may be labeled by enzymatic incorporation of radiolabeled (usually 32P or 33P) nucleotides or with nucleotides conjugated to haptens such as biotin for subsequent chemiluminescent detection. After probe hybridization and washing to remove nonspecific label, the hybridization signal is generally detected by exposing blots to X-ray film or phosphor storage plates, after prior incubation with chemiluminescent substrates if necessary. The resulting band identified by the probe indicates the size of the mRNA, and the intensity of the band corresponds to the relative abundance. Autoradiograph band intensities may be quantitated by densitometry, by direct measurement of hybridized radiolabeled probe via storage phosphor imaging or by scintillation counting of excised bands.

Because of the relative ease of Northern blotting, it has become the most widely used method for characterization of mRNA size and relative abundance. Of the techniques discussed here, Northern blotting is the only one which allows mRNA size determination, and therefore it has been a key method for detecting mutations that result in abnormal mRNA size. However, as a quantitative technique, Northern blotting presents several limitations resulting from the inability to control for the efficiency of RNA transfer and membrane binding, as well as ill-defined factors that affect kinetics of probe hybridization to nucleic acids on a solid support. In general, this technique should be considered semi-quantitative, and is most suitable for determining relative concentrations of mRNA species that occur in moderate to high abundance; for absolute quantitation or detection of rare mRNAs, other methods with greater sensitivity and lower background are desirable (discussed below). This technique also requires very high quality RNA preparations that demonstrate sharp 28S and 18S ribosomal bands after denaturing gel electrophoresis and ethidium bromide staining. Degraded RNA, evidenced by smearing of stained RNA and/or hybridization signal, or incomplete transfer of RNA to the membrane, will compromise the value of a Northern blot for measurements of mRNA abundance.

Northern blots are useful primarily to compare relative abundance of a particular mRNA species present in samples under different physiological or experimental conditions, rather than provide absolute quantitation. To compare individual samples for relative abundance of a particular mRNA, each sample on a blot must be hybridized with both the probe for the specific mRNA(s) of interest and a standard probe to detect an RNA species that should occur in equal abundance in all samples irrespective of the experimental treatment. Hybridization of the standard probe may be performed concurrently with the experimental probes if the two are of distinct size, or in a second round of hybridization after the blot has been stripped to remove the first probe. An example is shown in Figure 1, in which Northern blot analysis was used to demonstrate elevated expression of apolipoprotein (apo) A-IV mRNA in the intestine of mice carrying an apo A-IV transgene (Cohen et al. 1997). Hybridization was performed concurrently with probes for apo A-IV and apo A-I, a related mRNA for which expression levels should be similar in control...
and transgenic animals. Quantitation of the resulting hybridization signals using a phosphorimager revealed a fivefold elevation in apo A-IV mRNA in intestine of transgenic mice when normalized to apo A-I mRNA levels.

Several ubiquitously expressed mRNA species are used as standards for Northern blots, including β-actin, α-tubulin, β2-microglobulin, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and hypoxanthine-guanine phosphoribosyl transferase. Probes specific for 18S or 28S ribosomal RNA (rRNA) may also be used. The choice of an appropriate standard must be tailored to the specific experiment, as even the expression of ubiquitous transcripts and housekeeping genes may be altered in various experimental treatments and physiological conditions (Finnegan et al. 1993), at different stages in the cell cycle (Mansur et al. 1993), or among different cell and tissue types (Biragyn et al. 1994). Multiple studies have concluded that hybridization with 18S or 28S rRNA probes is a reliable control for sample normalization on Northern blots (Biragyn et al. 1994, de Leeuw et al. 1989, Finnegan et al. 1993). An alternative strategy to hybridization involves densitometric quantitation of ethidium bromide-stained rRNA after transfer to the membrane (Correa-Rotter et al. 1992). This technique eliminates the issue of variability in expression of housekeeping genes, as well as the need for a second round of hybridization. However, it is unlikely to find widespread use as it requires computer-assisted video densitometry of photographic negatives which have been made from the stained membrane.

Variations of the Northern blot, such as dot blots, slot blots and fast blots, have been developed in an effort to simplify blot preparation and improve quantitation. These techniques each involve applying an RNA sample (dot and slot blots) or cell extract (fast blot) directly to the membrane without prior size fractionation on a gel (reviewed in Costanzi and Gillespie 1987). With the use of a vacuum filtration manifold, samples are deposited onto a filter in a fixed array of dots or slots. After hybridization and detection by autoradiography, signals may be quantitated by densitometry, phosphorimaging or scintillation counting of filter spots. The relative ease of sample preparation and application permits the analysis of serial dilutions of each sample to allow comparison of the relative mRNA abundance between samples. Although this technique offers the advantage of being simpler to perform than a standard Northern blot, there is a compromise in the stringency with which the resulting data can be interpreted. Without size fractionation of the RNA, the results are blind with regard to the amount of hybridization signal produced by interaction with the target mRNA vs. hybridization to related sequences (such as members of a gene family) and nonspecific hybridization. Thus it is imperative to demonstrate via Northern blot that the probe used for dot/slot blot analyses is specific for the target mRNA without cross-hybridization or nonspecific hybridization to other sequences.

**Ribonuclease protection assay**

The ribonuclease protection assay (RPA) operates on the same principle as a Northern blot involving hybridization of a labeled probe to a target mRNA. However, in the RPA, hybridization takes place in a solution containing both a labeled antisense RNA probe and the target mRNA without prior gel fractionation or blotting (Azrolan and Breslow 1990, Sambrook et al. 1989) (see Fig. 2, upper panel). After incubation for several hours, unhybridized probe and sample RNA are enzymatically degraded and the remaining hybrids are electrophoresed through a denaturing polyacrylamide gel and visualized by autoradiography or phosphorimaging. Alternatively, the RNase-resistant hybrids may be precipitated and bound to filters for direct quantitation by scintillation counting (Melton et al. 1984). Furthermore, by performing titration reactions with unlabeled RNA transcripts corresponding to the mRNA sense strand, absolute RNA levels can be determined.

It is generally accepted that RPA offers at least 10-fold higher sensitivity than Northern blot analysis, allowing the detection of low abundance mRNAs (Sambrook et al. 1989). The sensitivity and specificity of the RPA can be attributed to the use of single-stranded RNA antisense probes which hybridize to a defined region of the target mRNA and are labeled to high specific activity (10⁹ cpm/µg). Single-stranded RNA probes are prepared using plasmid vectors containing specific sequences that serve as promoters for bacteriophage RNA polymerases, such as those derived from SP6, T3 and T7 phages (Lee and Costlow 1987). A number of such plasmids are commercially available, most of which contain two unique phage-specific promoters, one on either side of the polylinker sequences. A DNA insert corresponding to the mRNA of interest is cloned into the polylinker and RNA transcripts equivalent to either the sense or anti-sense strand are produced by choosing the appropriate phage RNA polymerase to initiate transcription (Lee and Costlow 1987). Radiolabeled antisense probes are produced by including a radiolabeled ribonucleotide (i.e., [³²P]-UTP) in the transcription reaction; a corresponding unlabeled sense RNA to be used as a titration standard may be transcribed from the opposite promoter by providing only cold ribonucleotides in the reaction. The size of the RNA transcript is controlled by predigesting the plasmid template with a restriction enzyme that cuts at a single site.
downstream from the promoter, thus serving to halt transcription due to polymerase runoff.

Several considerations apply to designing RPA probes that are suitable for accurate mRNA quantitation. RPA probe design requires previous characterization of the target mRNA so that the position of the probe within the transcript and the length of sequence to which it will hybridize have been established. If RPA products are to be analyzed by gel electrophoresis, the RPA probe should contain some terminal sequences that will not hybridize with the target mRNA, so that undigested probe can be distinguished from probe-RNA hybrids on the basis of size. This may be accomplished by including a portion of the plasmid multiple cloning site sequence within the antisense transcript that will not hybridize with the target mRNA, thus producing a 200-nt protected fragment that is distinguishable in size from undigested probe.

Probes may be multiplexed together in a single hybridization reaction, given that the sizes of products detected by individual probes do not overlap. Unlike the case with Northern blot hybridization, the size of an RPA product does not depend on the size of the target mRNA, but on the size of the RPA probe. As described above, probe size can be manipulated by altering the length of the transcription template, thus providing flexibility for designing probes that are compatible for multiplexing. If RPA products are to be analyzed by precipitation onto filters followed by scintillation counting, a difference in probe size is not adequate to allow distinction between hybrids of the internal control mRNA and the specific mRNA being assayed. A clever solution to this problem is the use of two different radionucleotides (i.e., $^{32}$P and $^{3}$H) for preparation of experimental and internal control probes (Melton et al. 1984). RNase-resistant hybrids derived from both probes can be quantitated on the same filter by choosing the appropriate parameters for scintillation counting.

The two major strengths of the RPA are sensitivity and the ability to determine absolute mRNA levels. As mentioned above, unlabeled sense RNA (cRNA) transcripts are easily synthesized from the same vectors used to prepare the antisense probes. The cRNA may be quantitated spectrophotometrically, serially diluted and then hybridized to an appropriate antisense probe to generate a standard curve against which RNA sample concentrations are determined. The analysis of
samples by RPA/filter precipitation has been shown to be sensitive to 5 pg of mRNA and linear to 120 pg, with a background of only 0.08% of input radioactivity (Melton et al. 1984). For absolute RNA quantitation the RPA/filter precipitation method is superior to the related DNA solution hybridization/S1 nuclease method because of its lower background, fewer enzyme artifacts and its use of RNA rather than single-stranded DNA as a standard, thus eliminating problems due to differences in RNA/DNA vs. RNA/RNA hybridization kinetics (Lee and Costlow 1987, Melton et al. 1984, Sambrook et al. 1989).

**Quantitative RT-PCR**

The unparalleled sensitivity of PCR has made RT-PCR the technique of choice to quantitate low abundance mRNA species and to detect mRNA in small numbers of cells. In theory, PCR should detect the cDNA derived from a single mRNA molecule, but in practice, 10 or more mRNA copies are required because of the relative inefficiency of the reverse transcriptase reaction required to convert mRNA to cDNA for subsequent amplification (Foley et al. 1993). Typically, very small amounts of total RNA (1 µg or less) are used for reverse transcription, and a fraction (1/20 to 1/50) of the resulting cDNA is used in the PCR.

In designing a quantitative RT-PCR assay, several variables that influence amplification must be determined and controlled. These include PCR cycling conditions (number of cycles, time and temperature of denaturation, annealing and extension steps, and ramp time between steps), concentration of reagents (dNTPs, oligonucleotide primers, reaction template and temperature stable DNA polymerase) and oligonucleotide composition (length, sequence, specificity and priming efficiency) (Innis et al. 1990, Volkenandt et al. 1992). Because of the sensitivity of PCR, very small amounts of genomic DNA contamination in an RNA preparation may serve as template for amplification and produce misleading results. To avoid this problem, PCR primer pairs should be designed such that the sequence for each primer occurs in a separate exon of the gene of interest; this allows the distinction on the basis of size between products resulting from amplification of cDNA from those which may have arisen from contaminating genomic DNA. It has also been recommended that RNA preparations be treated with DNase prior to cDNA synthesis, and that control reactions be performed in which reverse transcriptase is omitted (Dörnhöfer et al. 1995). Perhaps the most important factor that complicates the use of PCR for quantitation is the fact that the true exponential phase of the amplification reaction is of limited duration. As amplification products accumulate, the exponential phase eventually enters a saturation phase where products may approach similar levels irrespective of initial template concentration. Thus, for results to be meaningful, quantitative comparisons must be made during the exponential phase. Following is a discussion of various strategies for using RT-PCR to determine relative and absolute mRNA levels.

**Progressive analysis during the exponential phase—real-time PCR.** One strategy to ensure that PCR products are analyzed within the log phase of the amplification reaction is to examine products at progressive cycles during the reaction. This may be accomplished by manual removal of reaction products (Jaegle et al. 1996, Reue et al. 1997) or by the use of automated systems that permit real-time quantitative RT-PCR (Blok et al. 1997, Gibson et al. 1996, Heid et al. 1996). In its simplest implementation, aliquots are removed from the PCR every couple of cycles beginning at a point where product is undetectable (typically about cycle 20) and extending through the entire exponential phase. Products are then resolved electrophoretically and quantitated by densitometry, fluorescence or phosphorimaging.

This type of assay is useful for determining relative abundance of a target mRNA in samples of a given cell type that have undergone different treatments, or are in different cell types, as long as the abundance of the target sequence is normalized between the samples. For example, hormone-sensitive lipase mRNA is expressed at high levels in adipose tissue but at very low levels in monocytes. To allow direct comparison of hormone-sensitive lipase mRNA levels in these two tissues, cDNA from adipose tissue was diluted over a hundred-fold range to find the concentration at which PCR products from adipose and macrophages would appear at the same PCR cycle. We found that the appearance of product from undiluted cDNA prepared from primary monocytes and from the THP-1 monocyte cell line occurred at a similar PCR cycle as product from adipose cDNA which had been diluted 40-fold, indicating that hormone-sensitive lipase mRNA is expressed in monocytes at approximately 1/25 the levels in adipose tissue (Fig. 3; Reue et al. 1997). As a control, cDNA samples from adipose tissue, monocytes and THP-1 cells were diluted 30-fold and amplified with β-actin primers (Fig. 3).

Recent innovations have made it possible to follow the accumulation of PCR products in reaction vessels in real time without the necessity for manual collection of samples or post-PCR analysis and quantitation. Known as real-time PCR, this technique employs a fluorescent signal to report formation of PCR product as each cycle of the amplification proceeds, coupled with an automated PCR/fluorescent detection system (Heid et al. 1996). The assay is based on the use of a fluorescent probe that has both a reporter and quencher dye attached (Livak et al. 1995). The presence of the two dyes together leads to quenching of the reporter dye. However, when the probe anneals to the target cDNA sequence during PCR, the 5' nucleic acid activity of Taq DNA polymerase cleaves the reporter dye from the probe and a fluorescent signal is emitted. The resulting signal is automatically monitored at every cycle by a system that integrates the thermal cycler, reaction tubes with transparent lids, a laser for inducing fluorescence, and a detection and recording device. For absolute quantitation of target mRNA, an internal control template and corresponding control probe with a unique reporter fluorescent dye is included in each reaction tube (Gibson et al. 1996) (see below for further discussion of internal controls for PCR). Real-time quantitative PCR is extremely accurate, allows high throughput and is less labor-intensive than other methods available. However, at this point in time the cost of the integrated PCR/detection system is prohibitive for most labs which are performing quantitative PCR on a small scale. Thus, the other strategies for absolute quantitation of mRNA by PCR described below are still in most widespread use.

**Quantitative RT-PCR with endogenous standards.** Two general types of standards have been employed for absolute quantitation of target mRNAs by RT-PCR (reviewed in Foley et al. 1993, Raeymaekers 1995, Volkenandt et al. 1992). One involves the coamplification of an endogenously expressed standard RNA that is present in the same mixture as the target mRNA, much like the common practice of using housekeeping genes as normalization standards in Northern blot or RPA analyses. Experimental samples are normalized with respect to the endogenous RNA standard to allow quantitation of mRNA levels. The endogenous mRNA standard has the advantage of serving as a control for the extent of RNA recovery and integrity, as well as for sample-to-sample variations in reverse
transcription and PCR. As with the other mRNA quantitation techniques, the choice of a standard mRNA depends on the specific application and requires a demonstration that the standard RNA level is invariant under the conditions of the experiment.

However, with RT-PCR additional caveats for the internal RNA standard apply. The two primer pairs that will amplify the target mRNA and the internal standard must be compatible, ideally having similar annealing temperatures and no complementary sequences that could lead to primer artifacts. As indicated above, quantitation of PCR products will be valid only during the exponential phase of amplification. As a consequence, the initial concentrations of the endogenous RNA standard and the target mRNA must be similar so that their exponential phases of amplification will overlap. If this is not possible, the primer pair for the more abundant RNA may be added later in the amplification. To achieve reliable results, the amplification kinetics for both the internal standard and target mRNA must be carefully established by analyzing aliquots of trial PCR reactions every few cycles during amplification (Foley et al. 1993).

The utility of the internal standard RT-PCR method for mRNA quantitation is limited by two factors: that the absolute amount of the internal standard RNA typically is not known and the necessity that the target and control templates be coamplified using two separate primer pairs which may exhibit different amplification efficiencies. This strategy is therefore primarily useful for measuring differences in the relative concentration of a specific target mRNA in different samples and usually is not suitable for determining absolute amounts or for comparing levels of different RNA species. An exception to this latter condition occurs when mRNA transcripts for multiple members of a multi-gene family can be amplified using a single primer pair. This feature has been utilized for a quantitative comparison of expression levels of the GATA family of transcription factors during erythropoiesis (Foley et al. 1993), of $\alpha$, $\beta$, and $\gamma$-actin isoforms in the aorta (Raeymaekers 1995), and of multidrug resistance mRNAs MDR-1 and MDR-2 in multiple myeloma patients (Raeymaekers 1995). These kinds of applications are perhaps the most amenable to internal standard RT-PCR.

Quantitative RT-PCR with exogenous standards. The second quantitative RT-PCR strategy involves coamplification of an exogenous RNA standard which has identical primer binding sequences as those in the target mRNA template (Becker-Andreé and Hahlbrock 1989, Gilliland et al. 1990, Wang et al. 1989). Termed competitive RT-PCR, this method requires that several reactions be performed with a set amount of sample RNA and increasing known amounts of the standard. The competitor RNA standard is generally prepared by in vitro transcription and its concentration carefully determined. Since both the target and standard must compete for the same amplification primers, the amount of input target mRNA can be determined based on the standard concentration at which amplification products for target and standard are equivalent. Thus, unlike coamplification with an endogenous standard, absolute quantitation is possible. In addition, the use of a single primer pair eliminates many of the problems outlined above for endogenous standards. In particular, initial concentrations of standard and target RNA need not be similar and can differ up to two orders of magnitude (Bouaboula et al. 1992). Several investigators have assumed that the use of
the same primer pair for target and standard confers identical amplification efficiency and a constant ratio between target and competitor even beyond the exponential phase (Becker-André and Hahlbrock 1989, Gilliland et al. 1990, Siebert and Larrick 1992). However, other investigators caution against this assumption based on observations that the exogenous standard may have an “amplification advantage” over the target (Volkenandt et al. 1992) and that standard and target products accumulate with different efficiencies (Raeymaekers 1995, Wang et al. 1989, Weisner et al. 1993). It appears that the feasibility of using this technique beyond the exponential phase must be evaluated for each specific standard and target pair.

The successful design and implementation of a competitive RT-PCR assay requires consideration of the theoretical factors discussed above as well as several practical factors. The composition of the exogenous standard RNA has been a subject of much discussion in the literature (Bouaboula et al. 1992, Dirnhofer et al. 1995, Raeymaekers 1993 and 1995, Volkenandt et al. 1992, Zenilman et al. 1995). Most agree that in order to serve as a competitive standard RNA, the template must have identical primer sequences as the cellular target mRNA and similar, yet distinguishable, amplicon size. A common strategy for designing competitor templates is to insert or delete a restriction enzyme recognition site within the internal sequences of the template so that the products of the target and standard amplification can be easily resolved by electrophoresis after restriction digestion (Raeymaekers 1995). Although a general recommendation has been to keep the target and competitor amplicons a similar size to avoid differences in amplification efficiency, it appears that in some cases this may not be critical (Bouaboula et al. 1992). A solution to the laborious task of constructing a new standard template for each individual mRNA to be analyzed is to produce a single construct containing multiple PCR primer sequences (Bouaboula et al. 1992, Wang et al. 1989). Specifically, 5’ primers for a dozen or so target mRNAs are connected in tandem followed by the complementary sequences of the corresponding 3’ primers in the same order. The 5’ and 3’ primers may be separated by a linker region containing restriction enzyme recognition sequences, and the entire array is cloned downstream of an RNA polymerase promoter to allow production of RNA transcripts in vitro. This type of standard template has been employed to quantitate absolute levels of rare lymphokine mRNAs (Bouaboula et al. 1992, Wang et al. 1989).

The key for using competitive RT-PCR to perform absolute quantitation is the production of a log-log titration curve relating the PCR product ratio (standard/target) to the initial concentration of standard template added to the reaction (Gilliland et al. 1990, Raeymaekers 1993). This can be achieved by performing a series of reactions in which the amount of input target cDNA is held constant and the amount of standard competitor cDNA is increased over 2–3 orders of magnitude. By including a radiolabeled dNTP in these reactions, target and competitor PCR products can be quantitated by resolving on gels, isolating the fragments and scintillation counting. The PCR product ratio (standard/target) is then plotted on a log-log scale as a function of the input standard amount. This should produce a linear relationship with a slope equal to 1, which serves as a practical indication (but not proof) that both templates are amplified with the same efficiency (Bouaboula et al. 1992, Raeymaekers 1993). It has been pointed out that several published studies contain errors in titration curves used to quantitate RT-PCR (Raeymaekers 1993). Discrepant amplification efficiencies between the standard and target are most likely to show up when the amplification reaches the saturation phase and the PCR components decrease below levels required for optimal amplification. Thus, if an invalid titration curve is obtained (that is nonlinear or with a slope other than 1), a possible practical solution is to reduce the number of PCR cycles and/or the initial amount of template (Raeymaekers 1995). An unexpected source of error in generating titration curves is the use of agarose gels for quantitation of radiolabeled ethidium bromide-stained PCR products. A comparison of polyacrylamide and agarose gels for analyzing competitive PCR products has indicated that the for the same samples which produce a titration curve with a slope of 1 when analyzed on polyacrylamide gels, analysis on agarose produces a slope of significantly <1 (Bouaboula et al. 1992). This has been attributed to a 20-fold higher background in agarose compared to acrylamide (1000–2000 cpm vs. 50–90 cpm). One reliable approach appears to be quantitation of radiolabeled PCR products on dried polyacrylamide gels with phosphorimager scanning (Raeymaekers 1995).

**RNA/DNA quantitative PCR.** An alternative to the RT-PCR techniques with coamplification of either endogenous or exogenous RNA standards has recently been proposed (Zenilman et al. 1995). As this technique can be utilized for quantitation of either DNA or RNA, it has been named RNA/DNA quantitative PCR (RD-PCR). This technique utilizes genomic DNA present in total cellular nucleic acid preparations as an internal standard against which the concentration of the target mRNA is measured. This allows the determination of the number of mRNA copies per cell at a sensitivity 100-fold greater than for RPA (Zenilman et al. 1995). This is accomplished by carrying out the reverse transcription step using a hybrid primer which contains sequence complementary to the target mRNA at its 3’ end and sequence complementary to an intron of the corresponding gene at its 5’ end. This results in the production of a cDNA that is tagged for intron sequence; genomic DNA is not affected in the reverse transcriptase step. Competitive PCR is then performed in the presence of the intron-tagged cDNA and genomic DNA using the intron primer in combination with a 5’ primer derived from upstream exon sequence to simultaneously amplify the cDNA and genomic DNA. Conveniently, the amplicons generated from cDNA and genomic DNA will be of different sizes. As with RPA, a titration curve can be constructed with in vitro synthesized sense RNA corresponding to the target mRNA.

Although the RD-PCR method does not lend itself to the widespread applications as some other strategies for PCR quantitation, for some purposes it offers unique advantages. Unlike competitive PCR, it does not require construction of external standard templates, and by relating the mRNA concentration to that of genomic DNA, mRNA concentration per cell can be determined. Application of this strategy does, however, require that the genomic structure and some intron sequence is available. In addition, the number of gene copies per cell must be known, thus preventing the use of this technique with nonkaryotyped cell lines or tumors.

**Choice of mRNA quantitation method**

The choice of mRNA quantitation method for a particular application requires consideration of several factors (summarized in Table 1). Northern blot analysis is generally a logical first step in the characterization of mRNA expression as it allows visualization of intact mRNA and is the only technique discussed that provides information about mRNA size. Northern blot analysis also allows great flexibility, as the probe used
for hybridization does not require preparation with specific cloning vectors or primers. For applications in which sensitivity or absolute quantitation are of chief importance, however, RPA and RT-PCR are more suitable choices. These techniques are more readily applied to the analysis of mRNAs that have been previously characterized and sequenced, as they require production of specific vectors and primers for probe and control template preparation. In addition, RT-PCR requires careful optimization and titration to produce quantitative data. Thus, each of the three strategies discussed here provide specific advantages and limitations, and a combination of these techniques is often the best approach.

LITERATURE CITED


TABLE 1

Comparison of mRNA quantitation techniques

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<th>Property</th>
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