Real-time Polymerase Chain Reaction (PCR) is the ability to monitor the

Applied Biosystems offers two types of TaqMan probes:

TaqMan® probes (with TAMRA dye as the quencher dye)

TaqMan® MGB probes

Applied Biosystems recommends the general use of TaqMan MGB probes for allelic discrimination assays, especially when convent

The SYBR Green I dye chemistry uses the SYBR Green I dye to detect polymerase chain reaction (PCR) products by binding to double-stranded DNA formed during PCR. Here's how it works:

1. When SYBR Green I dye is added to a sample, it immediately binds to all double-stranded DNA present in the sample.
2. During the PCR, AmpliTaq Gold DNA Polymerase amplifies the target sequence, which creates the PCR products, or "amplicons."
3. The SYBR Green I dye then binds to each new copy of double-stranded DNA.
4. As the PCR progresses, more amplicons are created. Since the SYBR Green I dye binds to all double-stranded DNA, the result is an increase in fluorescence intensity proportionate to the amount of PCR product produced.

The advantages of the SYBR Green I dye chemistry are as follows:

It can be used to monitor the amplification of any double-stranded DNA sequence.
No probe is required, which reduces assay setup and running costs.

The primary disadvantage of the SYBR Green I dye chemistry is that it may generate false positive signals; i.e., because the SYBR Green I dye binds to any double-stranded DNA, it can also bind to nonspecific double-stranded DNA sequences.

Another aspect of using DNA binding dyes is that multiple dyes bind to a single amplified molecule. This increases the sensitivity for detecting amplification products. A consequence of multiple dye binding is that the amount of signal is dependent on the mass of double-stranded DNA produced in the reaction. Thus, if the amplification efficiencies are the same, amplification of a longer product will generate more signal than a shorter one. This is in contrast to the use of a fluorogenic probe, in which a single fluorophore is released from quenching for each amplified molecule synthesized, regardless of its length.

A Quantitation Assay is a real-time PCR assay. It measures (quantitates) the amount of a nucleic acid target during each amplification cycle of the PCR. The target may be DNA, cDNA, or RNA. There are three types of Quantitation Assays discussed in this chemistry guide:

DNA/cDNA quantitation
RNA quantitation using one-step reverse tr

(no template control) - A sample that does not contain template. It is used to verify amplification

For more information on using the comparative $C_T$ method for relative quantitation, please refer to User Bulletin #2: Relative Quantitation of Gene Expression (PN 4303859).

The standard curve method for absolute quantitation is similar to the standard curve method for relative quantitation, except the absolute quantities of the standards must first be known by some independent means.

The guidelines below are critical for proper use of the standard curve method for absolute quantitation:

- It is important that the DNA or RNA be a single, pure species. For example, plasmid DNA prepared from E. coli often is contaminated with RNA, which increases the $A_{260}$ measurement and inflates the copy number determined for the plasmid.
- Accurate pipetting is required because the standards must be diluted over several orders of magnitude. Plasmid DNA or in vitro transcribed RNA must be concentrated in order to measure an accurate $A_{260}$ value. This concentrated DNA or RNA must then be diluted $10^6$–$10^{12}$ -fold to be at a concentration similar to the target in biological samples.
- The stability of the diluted standards must be considered, especially for RNA. Divide diluted standards into small aliquots, store at –80 °C, and thaw only once before use.
- It is generally not possible to use DNA as a standard for absolute quantitation of RNA because there is no control for the efficiency of the reverse transcription step.

The absolute quantities of the standards must first be known by some independent means. Plasmid DNA and in vitro transcribed RNA are commonly used to prepare absolute standards. Concentration is measured by $A_{260}$ and converted to the number of copies using the molecular weight of the DNA or RNA.