Branched DNA (bDNA) Technology for Direct Quantification of Nucleic Acids: Design and Performance

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Introduction

The branched DNA (bDNA) assay represents a significant advance in the direct quantification of nucleic acid molecules for research and clinical applications. Other methods for the detection and quantification of nucleic acid molecules, such as polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA), involve molecular amplification of target nucleic acids followed by detection of amplified products. While these target amplification methods can be extremely sensitive, they suffer from two major disadvantages. First, it is difficult to obtain reproducible quantification since the target nucleic acid must be extracted and amplified in order to measure it. Second, numerous precautions must be taken to avoid false positive results caused by contamination of samples with PCR products and carryover from other specimens. A new departure from target amplification methods, the bDNA assay, directly measures nucleic acid molecules by boosting the reporter signal, rather than by replicating target sequences as the means of detection (Figure 1), and hence it is not subject to the errors inherent in the extraction and amplification of target nucleic acids. An ideal tool for nucleic acid quantification, the bDNA assay detects nucleic acid molecules at their physiological concentration, yields highly reproducible quantification, and eliminates false positives due to contamination.

The bDNA assay has been applied successfully in a number of clinical research areas, including the prognosis and therapeutic monitoring of patients with viral diseases. As a reliable method for quantification of viral load, bDNA assays have been developed to measure human immunodeficiency virus type 1 (HIV-1) RNA (Kern et al., in press; Pachl et al., 1995; Todd et al., 1995), hepatitis B virus DNA (Hendricks et al., 1995),

F. Ferré (ed.), *Gene Quantification*

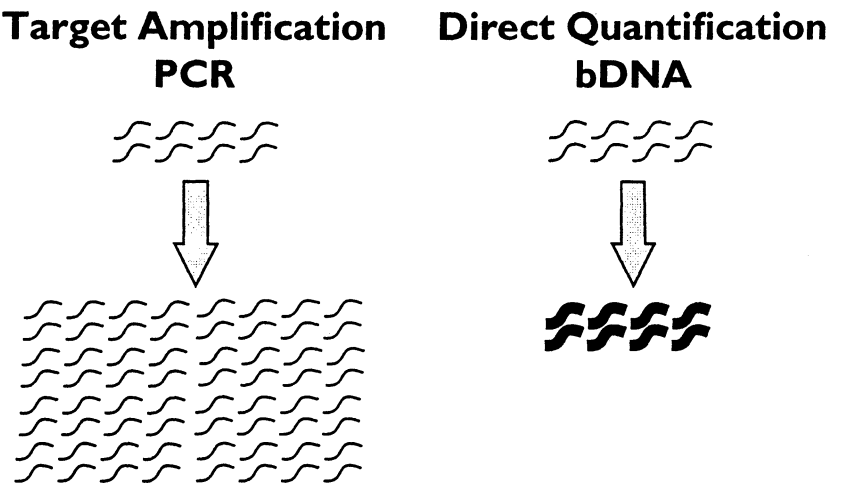

FIGURE 1. Comparison of target amplification methods to the bDNA assay for detection and quantification of nucleic acid molecules. Target amplification methods such as PCR first increase the number of target nucleic acid molecules to facilitate detection, then back-calculate to derive the original number of target nucleic acid molecules present. By contrast, the bDNA assay directly labels all target nucleic acid molecules and then counts them.

hepatitis C virus RNA (Davis et al., 1994; Detmer et al., 1996), and cytomegalovirus DNA (Chernoff et al., submitted; Flood et al., submitted). Yet, the potential applications of the bDNA assay extend far beyond viral nucleic acid quantification. Recent efforts have focused on quantification of cellular mRNAs. For example, the bDNA assay has been used for investigations into the concentration and intracellular location of specific cellular messages, splicing of intracellular messages, and expression of stress-induced genes for toxicology applications. Specific examples of novel applications of the bDNA assay are described in the chapter "Branched DNA (bDNA) Technology for Direct Quantification of Nucleic Acids: Research and Clinical Applications" in this book. With the development of bDNA assays for custom use, it is possible for researchers to use bDNA technology for a wide variety of specific research applications. This chapter describes the design of the bDNA assay for quantification of nucleic acid molecules and details the protocols and procedures for its use in a research or clinical laboratory. The development of reference standards for the bDNA assay is also presented, as well as specific examples illustrating the performance of the bDNA assay with regard to accuracy, sensitivity, and reproducibility.