Research Updates in Medical Sciences (RUMeS)
2013, Volume 1; Issue 1 page 27-29

Mini Review

Branched DNA: A Novel Technique for Molecular Diagnostics in Bone Studies

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Received 27-12-2012; Accepted 14-01-2013

How to cite:
Fathilah SN, 2013, Branched DNA: A Novel Technique for Molecular Diagnostics in Bone Studies. Research Updates in Medical Sciences 1(1) : 27-29

ABSTRACT

Recently, there are increasing applications of molecular techniques for molecular diagnostics in bone studies. Bone is a mineralized tissue containing an abundant matrix, which makes RNA isolation difficult. The most common technique used for molecular diagnosis in bone disease is reverse transcriptase–polymerase chain reaction (RT-PCR) sequence amplification assay. Currently, there is an increasing use of the branched chain DNA (bDNA) signal amplification technology. This article provides an overview of bDNA technology for molecular diagnostics in bone studies. Molecular diagnostic assays using bDNA technology for detection of gene expressions are sensitive, specific and reliable tools in the detection of genes involved in bone diseases such as osteoporosis.

1. Introduction

Currently there is increasing awareness among scientists, clinicians and general public of the costs and health care problems associated with osteoporosis, the most common metabolic bone disease. If the disease could be prevented or effectively treated, deaths, disabilities, and financial burden due to osteoporosis would be substantially reduced.

Recently there are evolvements in the molecular techniques uses for molecular diagnostics in bone study. These include RT-PCR (reverse transcriptase–polymerase chain reaction), real-time polymerase chain reaction and bDNA (branched DNA). The anti-osteoporosis mechanism of action of certain drugs or herbal products for the prevention osteoporosis could be elucidated by molecular studies [1,2]. This paper provides a brief review on the technique of branched DNA assay for molecular diagnostics in bone study. This technique is also compared with the conventional RT-PCR. The multiplex branched DNA assay involved incorporating the bDNA technology into the Luminex fluorescent bead-based platform through the use of cooperative hybridization, which ensures an exceptionally high degree of assay specificity [3].

2. Methods

The bDNA assay is a sandwich nucleic acid hybridization method that uses bDNA molecules to amplify signal from captured target RNA. In contrast with other techniques that rely on *in vitro* amplification of the target sequence (ie, PCR, transcription-mediated amplification, nucleic acid sequence–based amplification, and strand displacement amplification), the sensitivity of bDNA methods is achieved by signal amplification on the bDNA probe after direct binding of a large hybridization complex to the target sequence [4,5].These series of hybridization steps resulted in a “sandwich” complex of probes and target

sequence. These unusual synthetic oligonucleotides are composed of a primary sequence and secondary sequences which resulted in a branched structure extending from the primary sequence [6].

The initial step in a bDNA assay is to ensure that bone cells are lyses to release RNA. This is done by grinding the bone with hydrogen peroxide. By using the most recent third-generation bDNA assays, target-specific oligonucleotides (label extenders and capture extenders) are then hybridized with high stringency to the target nucleic acid [5]. The second step involves the target RNA capture. Probe Set design determines the specificity of the target RNA capture. This probe Set oligonucleotides composes of Capture Extenders, Label Extenders and Blocking Probe (CEs, LEs, BLs). They bind to the contiguous region of the target RNA and the CEs (Capture Extenders), by cooperative hybridization, selectively capture target RNA to the 96-well Capture Plate during an overnight incubation (Figure 1). The third step is the signal amplification, which is performed via sequential hybridization of Pre-Amplifier to Probe Set LEs (Label Extenders) and Label Probe to the amplifier. The number of LEs determines assay sensitivity. The forth and the last step is the detection of target mRNA present in the sample [7].

3. Discussion

The advantage of bDNA assays is that RNA is measured directly from the sample source, without RNA purification or enzymatic manipulation, thereby avoiding inefficiencies and variability introduced or by errors inherent to these processes [6].

The key advantage of bDNA is the high quality results of up to 80 different target genes obtained simultaneously in the same sample, in the same reaction. This enables researchers to quantitate the gene expressions of a large number of different genes and see their interaction or relationship with each other. The bDNA technique also allows the convenience of determining RNA gene expression directly from biological sample, without worrying about RNA contamination and degradation when handling the RNAs. In RT-PCR, preparation of purified RNA can be a difficult, running RT-PCR and obtaining consistent results can be challenging and requires a lot of practice and skill. The only limitation of bDNA is that it is designed for 96-well plate format, not in the tube format. Hence, this technique is not possible or economically sensible for a research which requires running a gene on a few samples, or a few genes on a few samples (Communication with Dr. Bui). Another advantage of bDNA technique is that it does not require reverse transcription or PCR amplification as in RT-PCR. The branched DNA technique is able to avoid biases associated with cDNA synthesis and enzyme inhibition or fluorescent dye incorporation during PCR amplification. It is also able to avoid sample losses as there are no target losses during RNA/DNA isolation. It has a much simpler assay workflow compared to RT-PCR. Yet, the sensitivity of bDNA is comparable to real time PCR and precisely quantify as little as 10% difference in gene expression. [8 -11].

In term of costing, bDNA is much cheaper than RT-PCR. The cost of bDNA technique is mainly to purchase the bDNA assay kit. It requires only about two days of running the bDNA assay which can be performed by

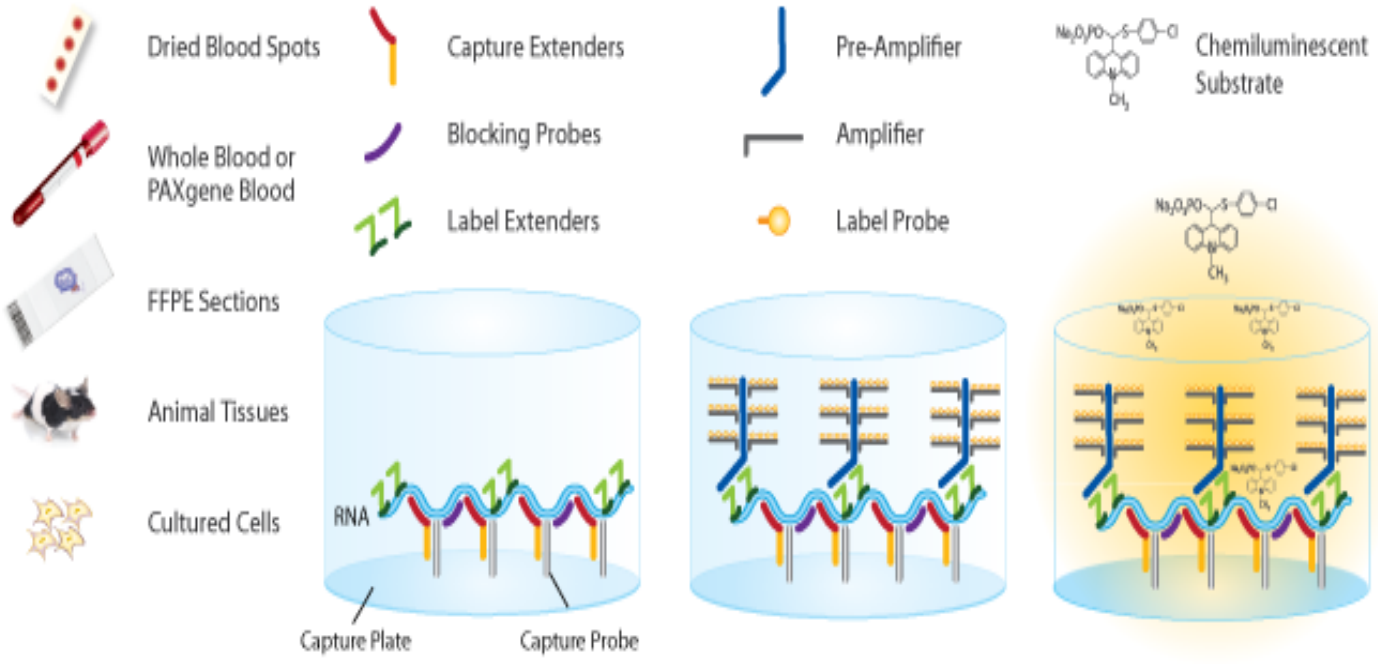

Figure 1: The mechanism of b-DNA. Figure was obtained with permission from https://www.panomics.com/products/gene-expression/single-plex-assay/how-it-works.

a single lab technician or researcher. Much time, energy and cost could be saved as this technique bypasses many steps such as RNA purification, reverse transcription and other laborious real-time PCR steps. For real-time PCR technique, the closest comparable technique to bDNA, the reseachers need to calculate the cost of obtaining the results for one gene per reaction per sample. This cost can vary from lab-to-lab as it is not always straight forward to obtain a good and sufficient total RNA for the first time. Besides that, the researcher will need to prepare and ensure all the consumables, tools and reagents and work bench surface to be RNAse-free. Next, the researcher needs to purchase the total RNA purification kit, Reverse Transcription kit and real-time PCR kit. There must be a good spectrophotometer available to determine the dsDNA concentration. The researcher will also need to optimize the real-time PCR reaction to obtain the best standard curve for quantitation. On the average, the time taken to obtain a good real-time PCR data from the sample and primer is one month for one target gene. However, the time taken may be longer for new users as this technique requires extensive optimization and troubleshooting to obtain reliable data [12].

The most expensive procedure of the RT-PCR technique is the trial-and-error involved in obtaining optimal RT-PCR results as it is not straight forward and easy to get them in the first run. RT-PCR technique is also expensive as it can only produce one result (data point) for one target gene per sample in one reaction compared to bDNA which can produce up to 80 different target genes (data points) per sample per reaction in one well. The time saving advantage of bDNA is very obvious and the quality of results obtained using bDNA is superb. The results can be compared from gene-to-gene because the results were obtained simultaneously in the same sample, in the same reaction [7].

4. Discussion

Branched DNA (bDNA) has progressed to be an alternative technique to RT-PCR (reverse transcriptase polymerase chain reaction) for molecular study in bone diseases. Branched DNA (bDNA) provides accurate, reproducible, time saving, highly sensitive automated laboratory tests as there is no RNA or DNA purification required. It is free from biases that usually occur with RT-PCR.

5. Acknowledgement

The author would like to thank Dr. Thuan D. Bui from i-DNA Biotechnology Pte Ltd for providing information and expert opinion on branched DNA (bDNA) technique.

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