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Application of Rna-Seq Technology in Gene Expression Measurement

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Abstract: RNA-seq is the most rapidly developed biomedical technology in recent years. It is a more accurate determination and analysis method at the transcription level, and has a wide range and important application in the field of passive plant research. In order to measure gene expression more accurately, most scientific research experiments will use RNA-seq technology. First, the RNA-seq technology uses reverse transcription to design a gene library, and then sequencing to screen genes. The process of analyzing the relationship between genes and functions using various analytical techniques. It is widely used by researchers mainly because of its high-throughput technology. This article introduces the application of RNA-seq technology in screening genes at home and abroad and the introduction of the experimental process, and makes a review.

1. Introduction

In order to screen the related genes of the tested species or the differentially expressed genes am ong species, the genes were measured by RNAseq technique, and then the

results were analyzed, then the differentially expressed genes were analyzed by GO

enrichment analysis and Kegg enrichment analysis, and the relationship between genes and funct ion was obtained. From sanger technology to RNAseq sequencing technologymore and more precisi on and refinement of human technology requirements, has been constantly concerned by researcher s. RNAseq, or transcriptome sequencing, is a highthroughput sequencing analysis of cDNA libraries that are reversetranscribed from

RNA such as Mrna, smallRNA, and NONcoding RNA to reflect their level of expression. The ai m is to use RNASeq technology to screen and analyze genes related to the species to be tested, it ha s been widely used in biological research, medical research, Clinical Research and Drug R & D and other research areas and plays an important role. This article reviews the application of RNAseq tec hnology in gene expression determination.

2. Introduction Of Rna-Seq

RNAseq is the most commonly used sequencing technology by researchers in recent years, beca use of its high throughput characteristics, known to the public. The idea is to reversetranscribe the Mrna into a CDNA, then attach the splice (known as an adapter) and place it into a sequencer to be sequenced. The specific method depends on different companies to carry out accurate measurement.

For example, the ILUMINA sequencing process, for example, take illumina's Truseq RNA library method (1) using a magnetic bead with a Poly (t) probe to hybridize with total RNA and absorb the Mrna with a Poly (a) tail, specific binding of Poly (t) and Poly (a) Tail Mrna. (2) the magnetic beads are recovered and eluted (3) the washed MRNA is resolved in a solution of magnesium ions, which break the RNA into short fragments. (4) the interrupted Mrna was reverse-transcribed by random primers (5) after the reverse transcription into the first-strand CDNA, then the second chain was synthesized as a double-stranded CDNA (6) A ”A” base was added to the two ends of the double-stranded CDNA, and a ”Y” type junction was attached to it. The test flow described above is a good description of the use of RNA-seq technology process.

3. Application And Analysis Of Rna-Seq Transcriptome Sequencing Technology

3.1 Screening Of Differentially Expressed Genes Based On Rna-Seq Technology

Screening of genes differentially expressed in Longissimus Dorsi muscle of Gaoligong Mountains pig and Duroc pig based on RNASeq technique, the data of transcriptome of Longissimus Dorsi from Gaoligong Mountains pig and DUROC pig were analyzed by edgeR software, and then the differentially expressed genes were analyzed by GO enrichment and Kegg enrichment. Go enrichment analysis is a common method, which is based on Wallenius noncentral, through differential expression gene GO database function annotation, the upregulated genes in the differentially expressed genes between Gaoligong Mountains pig and DUROC pig were analyzed for GO enrichment. In fact, significant enrichment analysis of GO function would produce GO functional entries, compared with the genomic background and the biology of differentially expressed genes, differentially expressed genes were significantly enriched, indicating whether they were significantly related to biological function. In the analysis, all differentially expressed genes are first mapped to each term in the Gene Ontology database, the number of genes in each term is calculated, and then the differentially expressed genes are the context of the entire genome. Then Kegg enrichment analysis was carried out and it was found that most of the genes differentially expressed in the GO and Kegg pathways were related to fat deposition. This paper briefly describes the process of sequencing and gene expression analysis of differentially expressed genes based on RNASeq technology, and lays a foundation for the molecular mechanism of fat deposition. A similar method was used in the screening and analysis of differentially expressed genes related to coat color in Tarim red deer, the difference was that the transcription level changes of 7 candidate genes were analyzed by real-time quantitative PCR (qrt-PCR) after GO and Kegg annotation analysis to verify the accuracy and reliability of the transcriptome sequencing results. Realtime PCR adds fluorescent group to PCR reaction system, ADDS fluorescent signal, uses this fluorescent signal to accumulate continuously to monitor the whole PCR process in realtime, finally uses the specific probe of staining or fluorescent marking. Here, Primer PREMIER5 software is used to design genetic quantitative primers. The results show that the real-time fluorescence results are basically consistent with the transcriptome results, and the accuracy and reliability of the results can be further verified by using this technique. The transcriptome analysis process of rhizome and fibrous root of Rhizoma Coptidis based on RNASeq technology is the same as the two experiments above, using the same Kegg technology, and the database integrates information of genomics, biochemistry and functional genomics, it can help researchers study genes and expression information throughout the network. In addition, Kegg provides a Javabased graphical interface for displaying gene maps, comparing genomes and expression maps, sequence comparisons, graphical comparisons, and path calculations. Thus, the Kegg database is one of the most powerful tools for in Vivo Metabolic analysis and metabolic network analysis. Therefore, Kegg analysis was used to determine the expression of all three genes, including 39 differentially expressed genes in the

transcriptome analysis of rhizome and fibrous root of Coptis Chinensis, and 21 related genes in the Kegg pathway, ten key enzyme genes were obtained, which indicated that the root of Rhizoma Copt idis was more complicated than that of rhizome of Rhizoma Coptidis. Several of the more typical ex periments mentioned above have demonstrated the good application of RNAseq technology in gene expression, and with the cooperation of bioinformatics technology, it will be very easy to reach the analysis conclusion.

3.2 Analysis Of Singlenucleotide Polymorphism And Variable Bases Of Genes Based On Rna-Seq Technology

Polymorphisms in DNA sequences caused by single nucleotide variations at the genome level are called Singlenucleotide polymorphism. Statistically, it accounts for almost 90% of known polymorphic sequences. And SNP polymorphism contains only one base change. This change may be caused by a single base shift or transposition, or by insertion or removal of a base. This is the most common type of Human genetic variation, and in most eukaryotes MRNA precursors produced by gene transcription are spliced in one direction to produce mature Mrna Molecules, so they are only translated into proteins. However, Mrna precursors of some genes undergo different splicing measures (selecting different splicing sites) t o produce different splicing isoforms. This process is called substitution splicing. RNA Alternative splicing is a more flexible method of regulating eukaryotic gene expression because it does not involve permanent changes in genetic information. In recent years, RNA transcriptome sequencing technology has developed rapidly, which can be used not only to accurately analyze gene expression level, but also to discover related functional genes, predict new transcripts, and analyze gene snps and AS. The number and distribution of snps in blastocysts of Yak cattle before and after vitrification were analyzed by R NASeq technique, and the number, event types and important differentially expressed genes (DEGs) of as were compared. The results showed that the number of snps of c/t type was slightly more tha n that of a/g type in fresh blastocysts, it can provide effective data and theoretical basis for further a nalysis and mining of the function of genes related to human embryos before and after cryopreservation.

3.3 Comparison Of Gene Expression Level Between Rna-Seq And Gene Chip

The RNA-seq technique still has advantages for gene chips. In principle, RNA-seq can determine the exact number of each molecule in a cell population, allowing direct comparison of results between experiments, and it can determine the level of RNA expression more accurately than a chip can. The RNAseq technique does not require a predesigned probe, so the data set is unbiased and provides a hypothetical experimental design. This type of NGS analysis is a powerful tool for fin ding and studying transcripts and mutations that are not possible with conventional microarray methods. Its unique low abundance of genes allows it to detect a hi gher proportion of differentially expressed genes than the expression chip. The RNAseq technique can achieve a dynamic range comparable to the real intr acellular transcripts of the gene chip, detect alternative splicing sites and new isoforms, and perform Ab initio analysis and reanalysis without reference to the genome, there are great advantages to analyzing data, and one of the great advantages of digital signals is that they can be used to detect complex

genetic mutations such as single base differences. The invention can also have a wider range of detection and application without designing a specific probe in advance. As a relatively new sequencing technology, the progress of science and technology has made the RNAseq technology more suitable for this era, and the requirement of human for precise measurement has also been gradually satisfied by RNA-seq technology.

4. Summary And Outlook

RNA-Seq is characterized by high throughput, high sensitivity, high resolution and no species restriction. It plays an important role in transcriptomics research and has obvious advantages over other traditional sequencing methods. It is widely used in differential gene expression analysis, Singlenucleotide polymorphism and variable base studies. With the development of technology and the saving of cost, the application becomes more and more extensive, and the software used to analyze the sequence result becomes more and more abundant. In this paper, the principles of the RNAseq transcriptome sequencing technology, the screening of differentially expressed genes based on the RNAseq technology, the analysis of Singlenucleotide polymorphism and variable bases based on the RNAseq technology and the comparison of gene expression level studies by the RNAseq microarray are summarized, there is a great understanding of the technology and its applications, which proves that the RNASeq technology occupies a very large role in these areas. These applications not only bring convenience to researchers, but also save a lot of time in the field of gene expression screening. It is widely used in the fields of gene mutation detection, gene expression research, adult and counterfeit detection of animal products and genetically modified food detection. In the future, with the progress of science and technology, the technology of detecting gene modified products by using RNAseq technology will be developed in the direction of higher quality, higher sensitivity, accuracy, automation and lower cost. We believe that in the near future, RNAseq technology will become more and more popular, more and more accurate.

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