Targeted Amplification of Mammalian Genome Sequences Using a Novel Deep Sequencing Approach: High Resolution Analysis of Mammalian Transcriptomes Using Designed Primers

Tech ID: 22381 / UC Case 2010-121-0

BACKGROUND

Sequencing based approaches of gene expression analysis generate millions of sequence tags, thus providing the dynamic range required to investigate genes of low abundance. Currently available digital gene expression analysis systems offer the potential for high-throughput transcriptomic measurements, however truly quantitative data are routinely not obtained. The most widely used RNA-seq protocol relies upon fragmentation of mRNA generating a library of uniformly distributed fragments of mRNA. This protocol requires large amounts of starting material (100ng of mRNA) limiting its application in many fields such as in developmental biology, where it is impractical to get such large amounts. Furthermore, this protocol maintains the relative order of transcript expression resulting in poor representation of low abundance transcripts at current sequencing depths. Multireads and biases introduced by transcript length and random hexamer primer hybridization further restrict reliable quantitation of low abundance transcripts for large mammalian transcriptomes. While random priming strategies amplify starting material (mRNA or cDNA) by exploiting hybridization and extension potential of hexamer/heptamer primers, they often result in low yield of good quality reads arising out of mis-hybridization of primers and primer dimerization. In a recent experiment, the inventors used a widely available sequencer to generate sequence tags via random priming strategy. Only 18% of the reads mapped uniquely to the transcriptome and low abundant transcripts were significantly under-represented because of poor dynamic range. Since many genes (signal transduction, transcription factors) are only expressed at relatively low levels, currently available strategies fall short in statistically quantifying these genes.

TECHNOLOGY DESCRIPTION

Researchers from UC San Diego have developed a patented cDNA expression profiling strategy, involving amplification of the majority (>80%) of mammalian transcriptome using a defined set of heptamer primers. The amplification protocol allows for efficient amplification of regions of interest from picograms of cDNA while minimizing mis-hybridization of primers and primer dimerization. The strategy reproducibly yields high levels of amplification necessary for sequencing-library generation from low starting material and offers a dynamic range of over five orders of magnitude in RNA concentrations. This invention reliably analyzes the mammalian transcriptome by employing ultra high throughput approaches and works on high or ultra high throughput sequencing platforms (e.g. Illumina's gene analyzer).

The inventor's system consists of software and novel primer sequences developed to hybridize to unique sequences identified in 74% of known genes. Care was taken to design this system to optimize amplification of target sequences that have low expression levels. The fragments produced are of sizes suitable for high-throughput sequencing and cover more than 90% of the transcriptome. This invention provides a platform for analyzing multiple samples at the same time thus reducing the operational cost.

ADVANTAGES

- First method that requires only picogram quantities of mRNA, while being sensitive to a large dynamic range of measurement concentrations, and thus capable of serving as an attractive method to study clinical samples from patients.
- Allows targeted amplification with the ability to exclude genes expressed at very high levels such as ribosomal genes, structure- and metabolism-related genes (e.g. genes that do not change their expression pattern in most of studies or represent the majority of the

CONTACT

University of California, San Diego
Office of Innovation and Commercialization
innovation@ucsd.edu
tel: 858.534.5815.

INVENTORS

- Subramaniam, Shankar

OTHER INFORMATION

CATEGORIZED AS

- **Research Tools**
  - Expression System
  - Nucleic Acids/DNA/RNA

RELATED CASES

2010-121-0

sequence tags). Their exclusion results in better representation of low abundance genes.

▶ Allows for the design of probes specific for known "hotspots" in the mammalian chromosomes, thus providing the ability to determine chromosomal abnormalities and structural variations. For example, this technology provides for the creation of unique primer kits to uncover single nucleotide polymorphisms in cancer or other disease-related genes.

▶ Useful for designing a defined set of primers to specific sequence regions of the transcriptome which may be mutated in certain genetic disorders, potentially useful in custom designed diagnostic kits for assessing the susceptibility of individuals towards specific diseases with greater accuracy than current approach: current approach consists of random sequence tag generation, resulting in a very low number of unique tags, and genes that are present in low abundance, thus limiting the statistical significance of any effort to quantify expression of low-abundance genes.

▶ Provides for the design of primers to specific sequence genes (e.g. Wnt signaling pathway) or phenotype (pluripotency related genes), thus expanding the scope of mRNA analysis to cover targeted functional pathways or disease mechanisms.

INTELLECTUAL PROPERTY INFO

Seeking commercialization partners for this patented technology.

RELATED MATERIALS

▶ Bhargava V, Head SR, Ordoukhanian P, Mercola M, Subramaniam S. Technical variations in low-input RNA-seq methodologies. Sci Rep. 2014 Jan 14;4:3678 - 01/14/2014

▶ Bhargava V, Ko P, Willems E, Mercola M, Subramaniam S. Quantitative transcriptomics using designed primer-based amplification. Sci Rep. 2013;3:1740. - 04/29/2013

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	9,920,367	03/20/2018	2010-121

University of California, San Diego
Office of Innovation and Commercialization
9500 Gilman Drive, MC 0910, ,
La Jolla,CA 92093-0910

Tel: 858.534.5815
innovation@ucsd.edu
https://innovation.ucsd.edu
Fax: 858.534.7345