A non-destructive method of quantifying mRNA in a single living cell

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OTHER INFORMATION

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BRIEF DESCRIPTION

The detection of levels of messenger RNA (mRNA), the molecule used by DNA to convey information about protein production, is a very important method in molecular biology. Current detection strategies, such as Northern Blotting and RT-PCR, require destruction of the cell to extract such information.

Researchers at the University of California, Irvine have developed a method to non-destructively assess mRNA levels in a single living cell.

FULL DESCRIPTION

Characterization of a particular gene requires a comprehensive analysis of mRNA expression both spatially (how it is distributed throughout the cell) and temporally (how its levels change over time). Once the mRNA levels are known, they can be used, for instance, to generate cDNA libraries, a very useful tool used to infer what are the results of a particular gene expression.

The most common techniques currently used present some disadvantages, such as low sensitivity, lack of flexibility, time consuming, and the necessity to physically destroy the cell to perform the assay.

Researchers at UC Irvine have modified an atomic force microscope to detect mRNA levels within a single living cell. Atomic force microscope probe tips are adapted to create a force that attracts mRNA molecules within the cell nucleus. The tip end is modified to match only the target mRNA within a pool of molecules within the cell nucleus.

The innovative technique is able to detect very low levels of specific mRNA sequences with less time to set up and acquire data (up to 2,500 mRNA copy numbers analyzed in less than 10 seconds). Additionally, the process maintains the cell intact, allowing for long-term studies of cell populations, and potentially, a diagnostic method for early detection of diseases.

SUGGESTED USES

Extraction of mRNA in living cells

- Research tools for basic biology studies, including constructing cDNA libraries

-Diagnostic tools for genetic-based human diseases, such as breast cancer

ADVANTAGES

Accessibly

§ The method is modified from a widely-used technology, Atomic Form Microscopy (AFM)

Highly specific and sensitive

§ Gene specific probes on the microscope tip are used to detect distinct mRNA sequence of interest

§ Capable of detecting very low copy numbers (10 molecules/cell)

Ultra-fast

§ Up to 2,500 mRNA copy numbers can be analyzed in less than 10 seconds

PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Issued Patent	8,365,311	01/29/2013	2010-809

STATE OF DEVELOPMENT

Working prototype:

§ Atomic force microscope (AFM) probes have been modified by immobilizing primers (20-30 base pair long)

§ Single cells are non-destructively analyzed using the probes by plating cells on glass cover slides or micropallet arrays were

Proof of concept:

§ Researchers have employed the technique to analyze the expression of breast cancer genes in transfected cells.

§ The target mRNA molecules were for GAPDH and Uts2r.

§ The AFM-based results were compared to conventional RT-PCR analysis.

RELATED MATERIALS

» Nawarathana, D., et. al. Targeting messenger RNA profiling of transfected breast cancer gene in a living cell. Anal. Biochem. 2011, 408, 342 - 01/15/2011

» Nawarathna, D., et. al. Selective probing of mRNA expression levels within a living cell. Appl. Phys. Lett. 2009, 95, 083117 - 08/27/2009

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