Technology Offer

Homogenous, TR-FRET-based method for measurement of RNA- and DNA-polymerase activity

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An innovative high throughput screening method for measurement of RNA- and DNA-polymerase activity at room temperature, based on time-resolved FRET.

Background

Polymerases are ideal targets for the drug therapy, for instance antibiotic, antiviral or cancer therapy. Therefore, modulation of polymerases is of high interest in the field of molecular biology. Modulators of polymerases may stimulate or inhibit their activity, but can also affect specific aspects of polymerase function. Traditionally, nucleotide polymerase activity assays are based on radioactivity and/or involve technical limitations. Known biochemical methods based on the incorporation of labeled nucleotides are heterogeneous and involve the risk of artifacts due to the interaction of the dyes with assay components. Furthermore, cell-based methods like qRT-PCR are based on expensive compounds and their throughput is limited.

Therefore there is an urgent need to provide methods for detection of nucleotide polymerase activity and especially methods of detection compounds that modulate nucleotide polymerase activity in an easy-to-apply, reliable, adaptable, safe and accomplishable-in-high-throughput-screening assay.

Technology

Scientists of the Max-Planck-Institute for biology of aging under Prof. Larsson and the Lead Discovery Center GmbH developed a method for the measurement of polymerase activity based on the detection of product formation. Using *in vitro* transcription and replication systems, the produced single strand mRNA or DNA, respectively, is recognized by sequence specific functionalized DNA oligonucleotides. These DNA-probes serve as proximity-based fluorescence donors and acceptors in a time-resolved fluorescent-resonance energy transfer (TR FRET) assay.

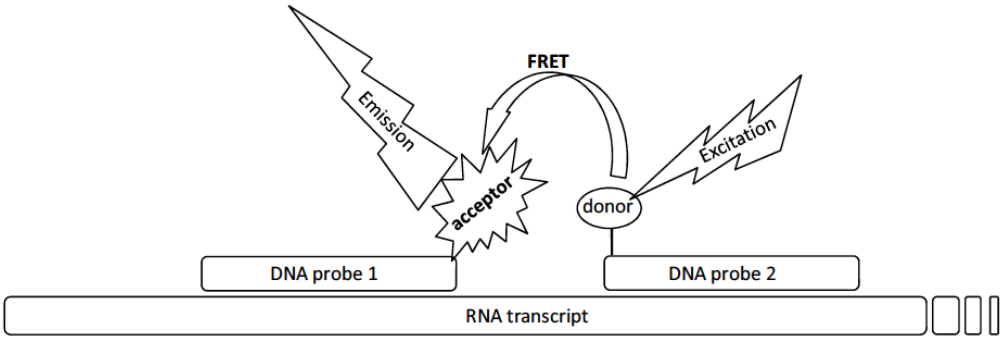

Figure 1: Schematic overview of the invented method of detection of nucleotide polymerase activity, consisting of specific, functionalized DNA-probes to serve as fluorescence donors and acceptors in a time- resolved fluorescence energy transfer (FRET) assay.

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This novel technology facilitated the establishment of homogeneous assay formats with working-volumes of <10µL, was found to be amenable to the use of 1536-well micro titer plates, with high signal-to-background ratio and very robust assay performance and reliability (z' >0,8). The assay has been tested and used for the identification of small molecule modulators of the enzymatic activity, as well as to assess selectivity of inhibitors against enzymes sourced from different organisms and of different cellular functions. Furthermore, the specific design of the detection method is conveniently adaptable to varying spectroscopic detection systems and different DNA-/RNA-polymerases, by the use of readily available custom DNA-synthesis.

Summary

In summary, the presented method was found suitable to measure RNA- and DNA-polymerase product formation by means of highly specific binding of DNA-probes and, thus, generating of a proximity-based, time-resolved fluorescence-resonance energy transfer (TR-FRET) assay. Furthermore, this method is adaptable to diverse polymerases and can be carried out at room temperature as high throughput assay.

We are looking for a licensing partner for this technology.
We will be pleased to share detailed information and scientific data.

Patent Information

European Patent Application EP 15 169 966.7 (filed May 2015)

Publications

Bergbrede T. et al. (2017): An Adaptable High- Throughout Technology Enabling the Identification of Specific Transcription Modulators. SLAS Discov. 22(4):378-386.

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