Docket #: S12-276

Method of RNA sample preparation from Ribonuclease-rich matrices

Researchers in Dr. Juan Santiago's lab have developed a quick simple method of RNA extraction that can be used with ribonuclease (RNase)-rich samples. RNA manipulations can be challenging as RNA is an extremely labile molecule that is easily hydrolyzed at elevated temperature and at either alkali or acidic conditions. Furthermore, RNA stability is significantly compromised by the abundance of RNases. Much care must be taken during RNA extraction and conventional methods are time-consuming, labor-intensive, tedious and involve toxic chemistries. This technology overcomes these limitations by providing a simple alkali-based extraction protocol with minimal manual steps.

Stage of Research
The inventors have used the method to extract 16S rRNA from bacteria suspended in whole blood and showed the rRNA was purified of PCR inhibitors and compatible with RT-qPCR. Furthermore, the inventors were able to detect the bacteria with a sensitivity of 0.03 bacteria per nanoliter of blood.

Applications

- RNA extraction from:
    - Biological fluids- blood, cerebral spinal fluid, etc.
    - Cell free matrices- serum, plasma, etc.
    - Homogenized tissue
    - Bacteria
    - Viruses
    - Combinations of the above samples
- Cell lysis
- Disruption of protein/nucleic acid complexes
- Irreversible inactivation of RNase in RNA suspensions

Advantages

- Simple protocol- no heating, precipitation, or buffer exchanges required
- RNA extraction performed entirely in aqueous solution
- Efficient cell lysis
- Rapid- total extraction time is less than 5 minutes
- Rapidly inactivates enzymes such as RNase
- Easily automatable
- Final RNA suspension can be compatible with:
    - PCR
    - Microarray hybridization
    - Standard RNA isolation/purification assays
    - Electric field based purification of nucleic acid including isotachophoresis

Publications

- U.S. Published Patent Application 20140057247, "METHOD OF PREPARING RNA FROM RIBONUCLEASE-RICH SOURCES".
- Rogacs, A., Qu, Y., and Santiago, J. G. Bacterial RNA Extraction and Purification from Whole Human Blood Using Isotachophoresis. Anal Chem. 2012 Jul 17;84(14):5858-63
- Gebel, E. RNA Gets Special Treatment To Remove It From Blood. C&EN. Web Date: July 11, 2012

Patents

- Published Application: 20140057247
- Issued: 9,057,673 (USA)

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