**INTRODUCTION**

Next generation sequencing (NGS) analysis of RNA isolated from clinical samples can reveal the presence of RNA viruses, expression levels of transcriptionally active DNA and RNA viruses, and identify correlations between host gene expression and viral levels, allowing for investigation of host cell changes that occur upon infection. Other current approaches to assess viral gene expression include targeted PCR methods, next generation sequencing (NGS), and metagenomic shotgun sequencing. However, NGS and targeted PCR methods are limited by sequencing, are limited by requiring previous knowledge of target sequences and by the level of multiplexing possible. While traditional RNA-Seq provides unbiased detection of all nucleic acids present in a sample, and therefore hypothesis-free data, it is limited by rather high input requirements (typically 50-100 ng of more total RNA). In addition, sequencing data of mixed viral/host RNA-Seq libraries is typically dominated by host reads. Therefore, without deep sequencing of each RNA-Seq library, viral reads may be missed altogether. Here we have a simple, robust, hypothesis-free RNA-Seq method that overcomes these challenges. As little as 50 pg of total RNA is converted to cDNA and amplified with Single Primer Isothermal Amplification (SPIA). After enzymatic fragmentation and NGS library generation, specific abundant and uninformative host transcripts are targeted for depletion, resulting in a significant reduction of the number of sequencing reads required to achieve viral detection as compared to traditional RNA-Seq methods. In this study, we present data demonstrating the utility of the Trio RNA-Seq workflow to detect viruses in various clinically relevant samples. The negative selection step to remove unwanted sequences, termed AnyDeplete, is fully customizable, allowing users to target any class of transcript for depletion within their final libraries. Additionally, the SPIA cDNA amplification method has been used extensively for sample preparation for downstream expression arrays, including in HIV virus discovery and characterization (Malboeuf et al., NAR 2013). The combination of these technologies is a powerful tool for viral analysis in clinical samples.

**TRIO RNA-SEQ TECHNOLOGY**

Trio RNA-Seq incorporates three innovative technologies

- **DNA Removal**
- **Amplification**
- **Transcript Depletion**

**METHODS**

**Sample Collection and RNA Isolation**

Nasal or nasopharyngeal samples were collected with a Nylon Flocked Dry Swab and Puritan UniTranz-RT Transport Systems transport media. RNA was isolated with 2μg direct-zol RNA miniprep. Samples were DNase I treated (in-column) and RNA eluted into DNase/RNase-Free Water. Yield ranged between 10ng and 700ng. Residual DNA contamination was removed using Zymo direct-zol DNA miniprep. Samples were DNase I treated and RNA isolated with Zymo direct-zol RNA miniprep.

**Reference Method**

Trio RNA-Seq: A Simple and Robust Method with Customizable Host Removal 1-Step End Repair & Enzymatic Fragmentation

**Trio RNA-Seq Enables Discovery**

**Increased Discovery of Clinically Relevant Bacterial Species in Trio RNA-Seq Libraries**

**Increased Coverage of Viral Genomes**

**Comparative Detection of Target Virus Between Trio and Reference Method**

**Compliance**

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**CONCLUSIONS**

1) The hypothesis-independent Trio RNA-Seq method exhibits high correlation to a target capture method for viral detection in clinical samples, while offering additional discovery opportunities for clinically relevant non-targeted virus and bacterial species.

2) The Trio RNA-Seq method produces libraries with a high level of viral genome coverage from minimal RNA input.

3) Removal of abundant host and uninformative transcripts improves detection of viral sequences.