Convenience and Power: Whole Blood Transcriptome Profiling using Microsampling

Analysis of gene expression in blood reveals molecular signatures of disease or drug response patterns. However, collecting blood samples presents significant challenges for biomarker monitoring and transcriptome profiling, often involving inconvenient clinic visits, large amount of blood draws and the instability of RNA in standard blood tubes. In this application note, we present an efficient method for transcriptome profiling from a drop of blood easily collected (at home) onto a convenient device (Mitra® microsampling technology, see Fig. 1). We find that Cellecta's DriverMap™ targeted RNA-Seq Expression Profiling assay can measure >18,400 protein-coding genes from one 30 µL microsampler with sensitivity and power comparable to those using standard methods. The sampled Mitra devices can be mailed to a laboratory for analysis after simple air-drying at room temperature, which will make blood transcriptome profiling much easier and more efficient, especially for repeated monitoring in clinical trials or disease-course studies.

Why whole blood transcriptome profiling?

Blood remains the easiest and most sampled human tissue. It is routinely used for numerous well-established biomarkers of health and disease (e.g., complete blood cell counts and chemistry assays). But for many disorders, we still need new biomarkers to improve diagnosis or prognosis, as well as to predict likely responses to drugs and other therapies. Blood is a logical and frequent target for biomarker discovery research because the molecules it contains often reflect disease pathobiology.

We focus on transcriptome profiling because of its many advantages. Since leukocyte pathobiology is central to most disease states, analysis of gene expression in circulating white blood cells provides a window into molecular signatures that accompany or even cause a disease or drug response pattern. Cellecta's DriverMap™ EXP assay uses a targeted amplification and sequencing approach to profile ~18.5K protein-coding genes from whole blood or PBMC samples. The targeted approach offers many advantages: 1) starts with total RNA; 2) eliminates the need to remove ribosomal RNA or deplete globin sequences; 3) minimizes sample handling, reducing variability and processing time; 4) simplifies bioinformatic quantitation and analysis. See https://cellecta.com/collections/drivermap-targeted-rna-sequencing for details.

Home blood microsampling can make transcriptome profiling and biomarker monitoring easy

Obtaining blood samples is a major barrier to biomarker monitoring along with the use of blood samples for transcriptome profiling. Typically, phlebotomy at clinics requires travel and effort by study subjects, or home visits by technologists, creating substantial practical and cost limitations. The Mitra® blood collection kits allow people to collect their own blood samples at home. With a simple lancet-induced method to produce a drop of blood from a fingertip, blood is absorbed quantitatively onto the Mitra® microsampling device. After air drying, the microsampling device can be mailed to a laboratory for extraction and analysis. The Mitra® microsampling device has been used extensively for many analytes in whole blood, including cytokines (McMahon et al), drugs (D'Urso et al), or metabolites (Taylor et al) but a suitable method for processing RNA was lacking.

The Mitra® device has recently optimized RNA sampling in whole blood (WB) by the incorporation of an RNA-stabilizing agent from GenTegra® into the VAMS® tips of the device (https://www.neoteryx.com/gentegra-rna-neo). Cellecta further optimized a protocol for WB transcriptome profiling with the DriverMap™ EXP assay using the new GenTegraRNA-NEO™ treated VAMS® tips and obtained promising, reproducible results, as detailed below.

Microsamples transcriptome profiling yields results similar to standard methods

We obtained venous blood from two healthy donors and analyzed gene expression profiles in the same samples either using the 30 µL VAMS® tips of the microsamplers in a Mitra® device treated with GenTegraRNA-NEO™, or adding Tempus RNA reagent at the

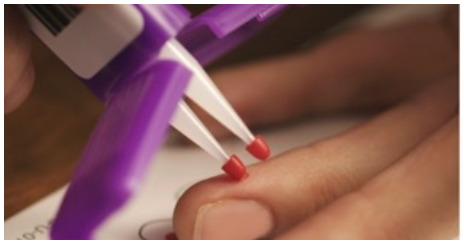

Figure 1

Mitra® Device absorbs a precise volume (10, 20, or 30 uL) of blood onto Volumetric Absorptive Microsampling tip.

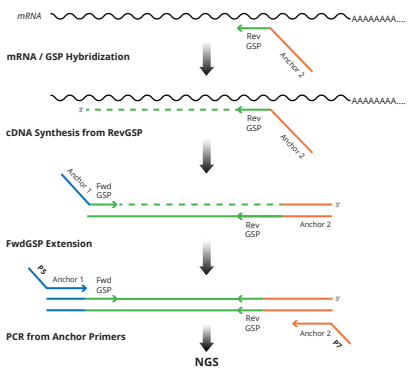

Figure 2

DriverMap™ Expression Profiling with Multiplex RT-PCR Workflow

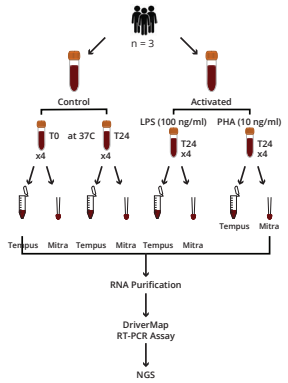

Figure 3

Design of Comparison Study

Continued on back

usual 2:1 ratio to 0.4 ml blood aliquots to stabilize and then isolate RNA using standard methodology (Qiagen). Replicate samples of blood were studied either immediately after collection (time zero) or after 24h of incubation with a panel of agents to compare the detection of differential gene expression after ex vivo whole blood activation (Fig. 3). For the microsamples, after air-drying at room temperature for 24h, RNA was extracted by immersing the tips in small volumes of Cellecta's optimized hybridization buffer, shaking on a heating block for 1h, and isolation of RNA using magnetic beads, with yields of approximately 50 ng RNA/tip. DriverMap™ target sequencing assay was then applied to each of the isolated RNA samples (Microsamples (MS) or Standard (Std)), followed by library preparation, sequencing, and bioinformatic analysis of the gene count data.

Comparison #1: Time zero

We tabulated the number of genes detected by the two methods in unstimulated whole blood at time zero as shown in Figs.4a & b. Using counts per million (CPM) normalization, the results show similar results for total genes detected using the two methods (~11K, with a slightly greater number of genes detected using the treated tips in the Mitra® devices). This number is consistent with the number of protein-coding genes detected in normal blood in numerous RNA-Seq studies. If we filter the data to remove genes with low/marginal counts and examine the number of genes with CPM > 1 or > 5 (not shown), the number of genes detected decreases but remains similar for both methodologies. Finally, we analyzed the small number of genes unique to one method or the other (~1.5%) that have a very low median CPM count, where known variability may push a gene to the other side of the > 1CPM filter. In summary, the comparison showed excellent results with both methods for measuring gene expression in normal whole blood.

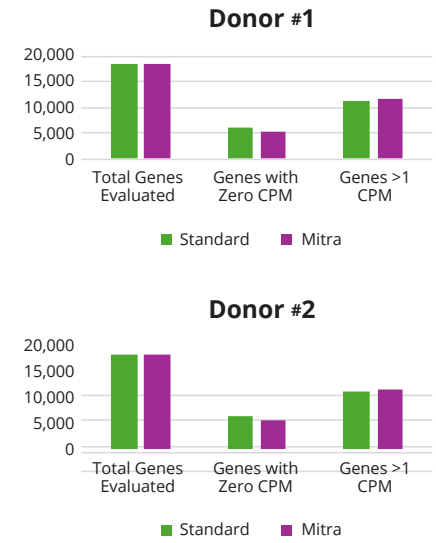

Figure 4 *Genes Detected at Time Zero across two donors show similar results for total genes detected using the two methods (Standard tubes and Mitra tips).*

Comparison # 2: Ex vivo activation for 24h

To evaluate the ability to detect differential gene expression in blood activated by inflammatory agents, we used a standard ex vivo whole blood stimulation protocol (Fig. 3). After 24h, replicate samples (n=4/group) of untreated blood or blood activated by the addition of stimulation agents (LPS 100 ng/ml, PHA 10 ug/ml, or anti-CD3&CD8 1 ug/ml) were sampled using the Mitra® VAMS® tips and the remainder of the blood (0.4ml) processed by addition of Tempus reagent and isolation of RNA as detailed above. We counted differentially expressed genes (DEGs) using criteria of a fold-change of > 2 and q < .01. The results show very similar numbers of up- and down-regulated genes in response to endotoxin (LPS) and phytohemagglutinin (PHA) in both normal donor samples (Fig. 5, similar results in donor #2 not shown). Comparison of the up-regulated genes shows high overlap in the same individual using MS vs Std methods and overlap of activated DEGs between the two individuals (not shown). Finally, to test the suitability of the Mitra® VAMS® tips for multi-omic analyses, we sampled the LPS-activated and control blood replicates using regular Mitra® VAMS® tips and evaluated cytokine protein levels using a multiplex immunoassay. As illustrated in Fig. 6, the protein results match the RNA data well, supporting the possible use of microsamples for both RNA and protein target analyses using customized immunoassay panels.

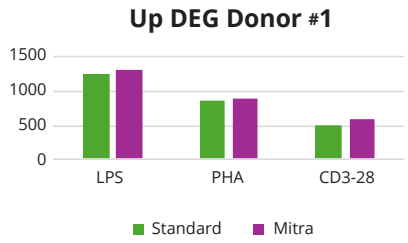

Figure 5 *Number of Up-regulated genes at 24 h after activation in response to LPS and PHA show similar results for using both methods (Standard tubes and Mitra tips).*

Summary

These data show excellent results using the VAMS® microsamplers in a Mitra® device treated with GenTegraRNA-NEO™ to profile whole blood transcriptomes using the DriverMap™ EXP assay. The optimized hybridization buffer and the multiplex RT-PCR technology allow the detection of >18,400 protein-coding genes in these microsamples. The results show similar detection of gene expression and up-and down-regulation in response to activation agents when compared to standard blood collection and RNA isolation methods. This innovative technology application allows convenient collection of blood microsamples at home. It greatly expands the ability of researchers to conduct large-scale transcriptomics and multi-omics (RNA, protein, and cytokine) studies for biomarker discovery applications.

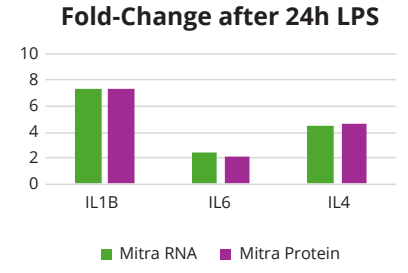

Figure 6 *Comparison of fold-change detected by Mitra at RNA or protein levels show that the microsamples can be used to detect both RNA and protein target analyses using customized immunoassay panels.*

For more information on DriverMap Targeted Expression Profiling (EXP) assay, email info@cellecta.com or call +1-650-938-3910

References:

1. McMahon R, Hill C, Rudge J, Herbert B, Karsten E. Stability of inflammation markers in human blood collected using volumetric absorptive microsampling (VAMS) under typical laboratory storage temperatures. Cytokine. 2023;171:156355. Epub 20230908. doi: 10.1016/j.cyto.2023.156355. PubMed PMID: 37690424.
2. D'Urso A, Locatelli M, Tartaglia A, Molteni L, D'Ovidio C, Savini F, Rudge J, de Grazia U. Therapeutic Drug Monitoring of Antiseizure Medications Using Volumetric Absorptive Microsampling: Where Are We? Pharmaceuticals (Basel). 2021;14(7). Epub 20210629. doi: 10.3390/ph14070627. PubMed PMID: 34209566 PMCID: PMCPMC8308594.
3. Taylor JM, Hughes AT, Milan AM, Rudge J, Davison AS, Ranganath LR. Evaluation of the Mitra microsampling device for use with key urinary metabolites in patients with Alkaptonuria. Bioanalysis. 2018 Dec;10(23):1919-1932. doi: 10.4155/bio-2018-0193. Epub 2018 Nov 6. PMID: 30412682.

CELLECTA

© 2024 Cellecta, Inc. All rights reserved. DriverMap is a trademark of Cellecta. All other trademarks are properties of their respective owners.