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Letter

Impact of Time Delay in Processing Blood Sample on Next Generation Sequencing for Transcriptome Analysis

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Dear Editor,

Detection of mRNA in blood samples is emerging as a potential biomarker of diagnosis or prognosis of disease [1, 2]. However, pre-analytical variations in mRNA expression can occur because of a delay in blood processing [3, 4]. In microarray-based transcriptome analysis, gene expression in blood cells was significantly altered by delayed blood sample processing (room temperature ≥ 2 hours) [3, 4]. There are no published studies of the effects of delayed blood sample processing on next generation sequencing for transcriptome analysis. This study was designed to address this..

Blood was collected from a healthy volunteer using 3 EDTA coated tubes. The process of RNA extraction began directly following blood collection, or after cold storage (4°C) for either 24 hours or 48 hours. Using a QIAamp RNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, RNA was extracted and its concentration was measured using a Quant-iT™ RiboGreen RNA Reagent (Invitrogen, Carlsbad, CA, USA). To calculate the RNA Integrity Number (RIN), the RNA 6000 Pico kitwas used and run on an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA, USA). The RNA was then sequenced on the GS Junior platform (Roche, Branford, CT, USA), according to the manufacturer's instructions.

Briefly, double-stranded cDNA was synthesized using the cDNA Synthesis System (Roche Diagnostics, Mannheim, Germany), was fractionated into 600-900 bp fragments via nebulization by compressed nitrogen gas, and then cDNA fragments were ligated to 2 adaptors for 3' and 5' ends. Following purification and quantitation, DNA libraries were used for emulsion PCR (emPCR) and bead enrichment. Sequencing was carried out using the GS Junior Titanium Sequencing Kit (Roche Diagnostics). Contig assembly was performed using CLC Genomic Workbench 6 (CLC bio, Aarhus, Denmark).

To evaluate the effects of delayed blood processing on RNA-seq analysis, concentrations of RNA, RIN, and the number of contigs were assessed from the same volume of blood prepared directly following collection, or after cold storage (4°C) for either 24 hours or 48 hours (Table 1).

The concentration of RNA was much lower in blood sample (13 ng/μL) that was stored at 4°C for 48 hours compared to the blood sample (34 ng/μL) that was prepared immediately.

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A decrease in RIN value (≤ 20%) was observed for cold storage delayed processing and the number of contigs gradually reduced by ≥ 40%.

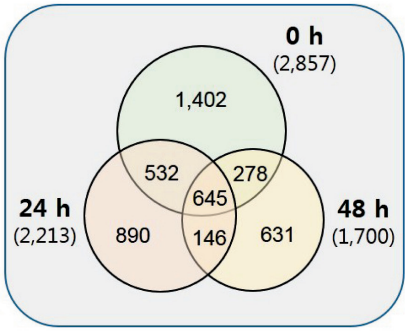
The number of transcripts, annotated with contigs via an expressed sequence tag (EST) database, was 2857 in the blood sample prepared immediately and for cold stored samples, 2213 for a 24-hour delay and, 1700 for a 48-hour delay in processing. The number of transcripts also gradually decreased by ≤ 40% according to processing delay.

Amongst the transcripts, 146 transcripts were detected in both blood samples stored at 4°C but not in the blood sample prepared immediately. These included genes associated with signal transduction (e.g. IMPA2, ABR, MAP2K1, RASGRP2, RIN3), transcription (e.g. PHB, GATAD2A, MDM2, RNF25, S100A12), and cell cycle arrest (e.g. CCDC88B, CCR2, PYCARD) (data not shown). A few biomarkers for diseases were differentially expressed by delayed processing of blood samples; mRNAs of C-C chemokine receptor type 2 (CCR2) and C-X-C motif chemokine receptor 1 (CXCR1) were only detected when the processing of blood samples was delayed, but not in the blood sample prepared immediately after blood collection. These genes are putative mRNA biomarkers in peripheral blood for progression or severity of hepatocellular carcinoma [5] and coronary artery disease [6], respectively.

These findings indicate that the processing condition of blood samples may affect the diagnosis of disease using mRNA biomarkers. Contrary to this result, CCR2 expression in microarray data has been shown to decrease with delayed processing. The difference between studies supports findings that pre-analytical variations can depend on the assay method [7].

In conclusion, the impact of delayed blood processing on next generation sequencing data for transcriptome analysis has been identified. This study has found that the quantity and quality of RNA in a blood sample was sensitive to time from collection to processing. The number of detectable transcripts decreased and expression patterns of transcripts including putative mRNA biomarkers, altered according delayed processing. Our study suggests that a blood sample with a longtime-delay from blood collection to processing, should not

Table 1. Effects of a delay in blood sample processing on RNA-seq analysis.

		Delay time		
		0 h	24 h	48 h
RNA Quality	RNA concentration (ng/μL)	34	32	13
	RNA Integrity Number	10	8	8
Contig (NGS data)	N75 (bp)	503	502	486
	N50 (bp)	614	582	537
	N25 (bp)	955	905	801
	Minimum (bp)	23	31	32
	Maximum (bp)	4,097	4,457	5,055
	Average (bp)	633	615	572
	Total length (bp)	4,160,391	3,194,787	2,188,883
	Contig count	6,577	5,191	3,829
Venn diagram (NGS data)	Gene Count			

bp = base pairs; NGS = next generation sequencing.

be used for transcriptome analysis for research or diagnostic purposes. However, further studies with more volunteers are necessary.

Conflicts of Interest

The authors declare no conflicts of interest.

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