BIOPRESERVATION AND BIOBANKING
Volume 8, Number 3, 2010

DOI: 10.1089/bio.2010.0009

Gene Expression Profiles from Peripheral Blood Mononuclear Cells Are Sensitive to Short Processing Delays

Michael G. Barnes,[1,2] Alexei A. Grom,[1] Thomas A. Griffin,[1] Robert A. Colbert,[1,*] and Susan D. Thompson[1]

In the analysis of peripheral blood gene expression, timely processing of samples is essential to ensure that measurements reflect *in vivo* biology, rather than *ex vivo* sample processing variables. The effect of processing delays on global gene expression patterns in peripheral blood mononuclear cells (PBMCs) was assessed by isolating and stabilizing PBMC-derived RNA from 3 individuals either immediately after phlebotomy or after a 4 h delay. RNA was labeled using NuGEN Ovation labeling and probed using the Affymetrix HG U133 Plus 2.0 GeneChip®. Comparison of gene expression levels ($\geq$2-fold expression change and $P < 0.05$) identified 307 probe sets representing genes with increased expression and 46 indicating decreased expression after 4 h. These differentially expressed genes include many that are important to inflammatory, immunologic, and cancer pathways. Among others, *CCR2*, *CCR5*, *TLR10*, *CD180*, and *IL-16* have decreased expression, whereas *VEGF*, *IL8*, *SOCS2*, *SOCS3*, *CD69*, and *CD83* have increased expression after a 4 h processing delay. The trends in expression patterns associated with delayed processing were also apparent in an independent set of 276 arrays of RNA from human PBMC samples with varying processing times. These data indicate that the time between sample acquisition, initiation of processing, and when the RNA is stabilized should be a prime consideration when designing protocols for translational studies involving PBMC gene expression analysis.

Introduction

Biobanking practices require support from evidence-based biospecimen science to ensure that findings are due to *in vivo* biological differences rather than *ex vivo* influences. This is especially important in global gene expression studies such as those using Affymetrix GeneChips® that monitor the expression level of ~47,000 transcripts from a single sample and can be highly sensitive to preanalytical variability.

Although several studies have examined the effect of preanalytical variables on global gene expression in solid tissues[1–3] (among others), much less is known concerning the effect of delayed isolation of peripheral blood mononuclear cells (PBMCs). In one study comparing aliquots of PBMCs processed immediately or after overnight shipping to a single processing center, 2034 out of 6414 genes were found differentially expressed.[4] Independently, Affymetrix, Inc., identified extensive expression pattern changes in PBMC samples due to overnight processing delays.[5]

In this study we identified gene expression changes in PBMCs related to processing delays as short as 4 h. These patterns were also apparent in an independent set of 276 arrays with variable processing times.

Materials and Methods

Subjects

Blood samples from adult volunteers were used to examine the processing time variable directly. In addition, data were available from a large, multicenter, gene expression study related to juvenile idiopathic arthritis (JIA). Patients in the JIA study were followed for up to 2 years and blood samples were collected at up to 10 time points for each patient. Blood was collected after informed consent.

Sample processing

Sample processing has been detailed elsewhere.[6–8] Briefly, peripheral blood was collected in acid citrate dextrose (ACD) tubes, and PBMCs were isolated over Ficoll gradient (Ficoll Paque™ Plus; GE Healthcare, Piscataway, NJ) and put into TRIzol® reagent (Invitrogen, Carlsbad, CA) for 5 min at room temperature before slow cooling (−1°C/min), and storage at −80°C. The time between phlebotomy and start of processing is defined as the "processing delay." The time between phlebotomy and storage at −80°C is defined as "time to freezing" (TTF). RNA was extracted and purified using

[1]Division of Rheumatology, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio.
[2]Cincinnati Biorepository Core Facility, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio.
*Present address: National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland.

RNeasy columns (Qiagen, Germantown, MD). cDNA was prepared using NuGEN Ovation kit version 1 (NuGEN Technologies, San Carlos, CA) from 100 ng of RNA and assayed using Affymetrix HG U133 Plus 2.0 GeneChips (Affymetrix, Santa Clara, CA). The timecourse microarray dataset has been deposited in the Gene Expression Omnibus at the National Center for Biotechnology Information (NCBI) and is accessible through GEO Series access number GSE21039.

Statistical analysis

Data were preprocessed in GeneSpring GX 7.3.1 using robust multichip analysis.[9] Probe sets representing differentially expressed genes were defined as those with $\geq$2-fold expression difference and $P < 0.05$. Hierarchical clustering used Pearson correlation for probe sets and distance correlation for samples. To allow comparison to probe sets reported in the literature using an earlier version GeneChip, a conversion was performed using the GeneSpring 7.3 "translate" function.

Results

Short processing delays alter gene expression

Three tubes of blood were collected from a single venipuncture from each of 3 donors, and PBMCs were isolated after a 0 h (T0), 2 h (T2), or 4 h (T4) delay at room temperature. Comparing GeneChip signal intensity values between T0 and T4 identified 353 probe sets detecting differentially expressed genes (select probe sets in Table 1, and full list in the Appendix. Hierarchical clustering of the samples using these probe sets completely segregated the samples into their respective processing-time category (Fig. 1A).

Table 1. Selected Genes of Immunological Importance

		Fold change[b]	
Probe ID[a]	*Gene symbol*[a]	*2 h/0 h*[c]	*4 h/0 h*[d]
207794_at	*CCR2*	0.63	0.38
206991_s_at	*CCR5*	0.53	0.39
223751_x_at	*TLR10*	0.64	0.45
216379_x_at	*CD24*	0.55	0.45
206206_at	*CD180*	0.61	0.47
209828_s_at	*IL16*	0.67	0.47
223583_at	*TNFAIP8L2*	0.60	0.47
207907_at	*TNFSF14*	0.62	0.49
227697_at	*SOCS3*	1.71	2.14
218881_s_at	*FOSL2*	1.69	2.16
209795_at	*CD69*	1.60	2.23
230170_at	*OSM*	2.26	2.29
218311_at	*MAP4K3*	1.55	2.40
201465_s_at	*JUN*	1.33	2.41
203373_at	*SOCS2*	1.53	2.50
202644_s_at	*TNFAIP3*	2.30	2.79
211506_s_at	*IL8*	2.27	3.23
217738_at	*PBEF1*	2.04	3.31
204440_at	*CD83*	2.20	3.47
210512_s_at	*VEGF*	1.89	3.48

[a]Affymetrix U133 Plus 2.0 GeneChip® probe set IDs and annotations.
[b]Fold change as ratio of geometric means.
[c]Expression at 2 h/expression at 0 h.
[d]Expression at 4 h/expression at 0 h.

Response to overnight processing delays is different

In a previous report, Baechler et al. identified 2155 probe sets (2082 HG U133 Plus 2.0 equivalents) measuring differentially expressed genes between peripheral bloods processed to PBMCs immediately and those bloods shipped by overnight courier before processing.[4] Understanding that there were significant experimental differences between the 2 studies, the current list of 353 probe sets was compared and showed only 62 probe sets (17%) in common. Although there were clusters of genes that appeared different in samples with delayed processing, the 2082 probe sets were unable to distinguish the current samples according to processing time (Fig. 1B).

Validation in a larger cohort

To determine whether the processing time signature would be apparent in samples that exhibit variation in gene expression for other reasons, the expression pattern (shown as the average of the expression levels for observation purposes) was examined in an independent group of 276 arrays (Fig. 2). The PBMC samples were obtained either from healthy controls or from patients with JIA and TTF ranged from 60 to 315 min. Although these samples showed some variation (possibly indicating biological variation between samples), trends of expression changes due to processing time were evident (Fig. 2). The trend was apparent in samples from each clinical site and each disease subtype showing that the processing time effect is independent of either clinical site or disease state (data not shown).

Discussion

Many studies have reported gene expression differences in PBMCs without providing details of sample processing procedures. This study highlights the importance of considering processing delays since periods as short as 4 h can have significant effects on gene expression. While additional studies with independent samples are necessary to define specific processing gene expression signatures, it is clear that processing delays must be considered in experimental design and data interpretation.

This study was not designed to determine the specific cause of the identified gene expression changes although several explanations can be hypothesized. Phlebotomy itself can have an influence on lab results obtained from blood samples.[10] Regarding storage of samples after phlebotomy, a previous study looked at the effect of room temperature storage of serum and plasma.[11] Within 4 h after phlebotomy, the environment of the plasma and serum sample changed significantly, which would cause biological stresses. Glucose levels dropped, lactate increased, pH decreased, and hypoxia were identified by a decrease in pO_2 and an increase in pCO_2. Although this study did not examine the effect of blood processing delays, it can be expected that similar effects would be encountered. Even the simple preanalytical variables of the additive in the collection tube (eg, ACD in this study) or interaction of cells with the surface of the tube itself (a significant change from the vasculature of the donor) for several hours may cause the identified gene expression changes.

Proteins derived from the genes listed in Table 1 are important in inflammatory, autoimmune, and cancer pathways.

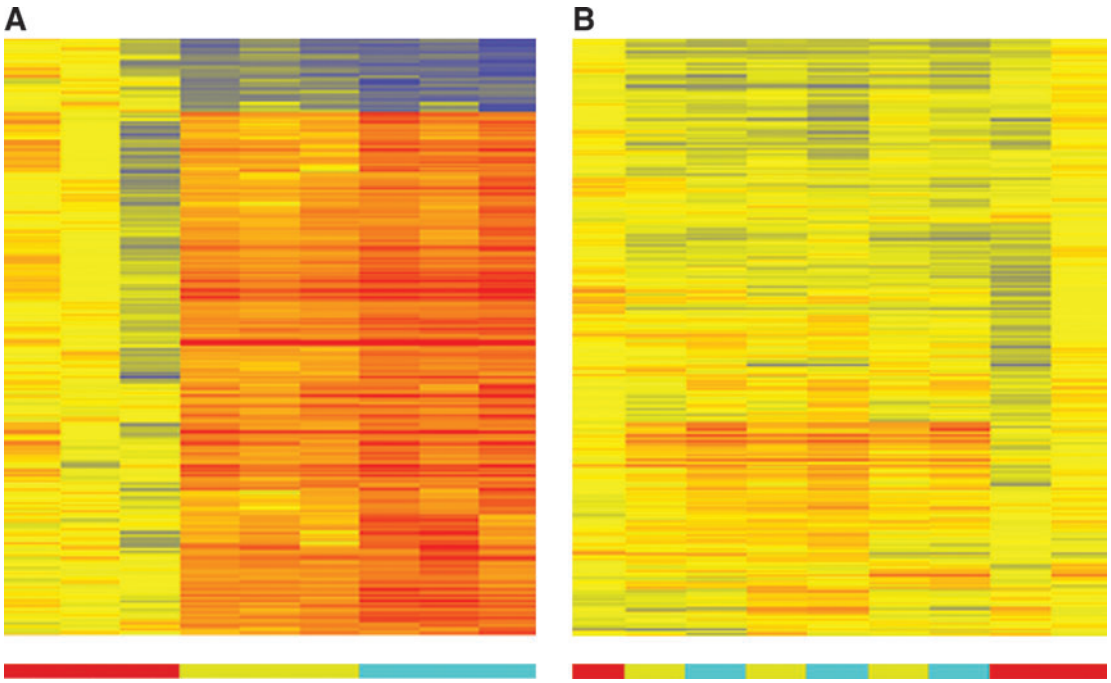

FIG. 1. Processing effects of gene expression signatures. (**A**) Samples clustered according to 353 probe sets identified ($P < 0.05$ and fold-change $\geq$2-fold) representing genes differentially expressed between samples processed immediately or after a 2 or 4 h delay. (**B**) Samples from this study were clustered according to gene expression data related to 2082 probe sets identified by Baechler et al. as differentially expressed after overnight shipment of peripheral blood before RNA extraction.[4] Each column represents 1 sample and each row represents 1 probe set. In the heatmap, expression levels are indicated by color (yellow, median; red, increased; blue, decreased). Bar at the bottom indicates class membership: red, immediate processing; yellow, 2 h delay; blue, 4 h delay. See online article at www.liebertonline.com for color figure.

Vascular endothelial growth factor (VEGF) is a prototypical angiogenic factor,[12] and anti-VEGF has become the standard treatment for many tumor types.[13] CCR2, CCR5, and IL-8 are chemoattractants that activate and recruit immune cells to sites of infection and inflammation. Antagonists to CCR2 have been suggested as therapy for a variety of inflammatory diseases as well as obesity and pulmonary disease.[14] The CCR5 (also called CD195) antagonist, Maraviroc, is currently approved for treatment of HIV infection.[15] SOCS2 and 3 are key negative regulators of cytokine signaling.[16] TLR10 and CD180 (RP105) are involved in toll-like receptor signaling. The fact that these genes have variable expression attributable to processing delays emphasizes the importance of attention to preanalytic variables in sample collection and processing, a fundamental component of biospecimen science.

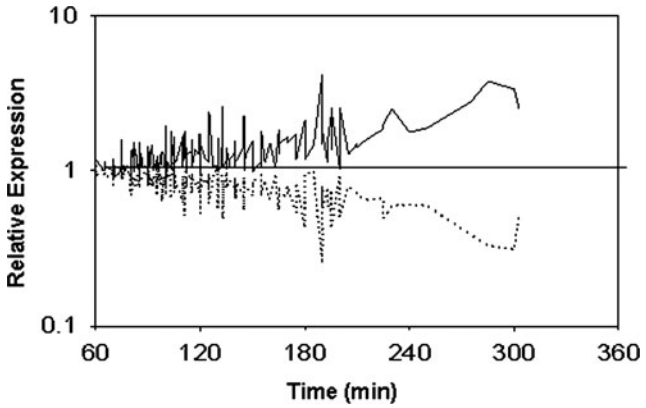

FIG. 2. Average expression levels of time-sensitive probe sets. In a pilot experiment, 307 probe sets were identified with increased expression and 46 with decreased expression after a 4 h processing delay. Average expression of probe sets with increased (solid line) or decreased (dotted line) expression is shown in 267 independent samples. Samples are arranged according to time to processing.

Various strategies can be employed to address possible changes caused by processing delays. The method we chose to reduce the impact of this variable was to remove samples with extended processing times (>4 h) from analysis.[6–8] As an alternative, time-sensitive genes may be removed from consideration.[17] Both of these methods have the benefit of simplicity although specific cutoffs are arbitrary. A disadvantage of this approach is that time-sensitive genes might also be involved in pathogenic processes. An interesting, but untested, method would be to delay processing of all samples until a specified time postphlebotomy (eg, 2 h) to reduce this variability. Alternatively, statistical approaches such as linear modeling may be employed using processing time as 1 variable.

To avoid the effect of variable processing time, technologies that stabilize samples instantly have been developed. These methods collect whole blood, including neutrophils that tend to vary in number more than other cellular components, and metabolically active immature red blood cells. Together, these components may overshadow more biologically relevant PBMCs. Additionally, there are issues with the so-called globin effect, where excessive amounts of globin mRNA derived from the reticulocytes present in

whole blood interfere with the microarray analysis as seen by decreased present calls.[5] It is unclear if this occurs due to inhibition during the labeling or probing steps of the assay. Newer labeling systems may help overcome this issue (as indicated by recovery of detected genes; unpublished data).

It is evident from this study that processing delays affect gene expression patterns obtained from PBMCs in a very short period. It is, therefore, important for this variable to be measured so that its effect can be considered in data interpretation.

Acknowledgments

This work was supported by the NIH/National Institute of Arthritis and Musculoskeletal and Skin Diseases (Grants P01AR048929, P30AR047363, and P60AR047784), the Cincinnati Children's Hospital Research Foundation, and the Ohio Valley Chapter of the Arthritis Foundation.

Author Disclosure Statement

No competing financial interests exist.

References

1. Dash A, Maine IP, Varambally S, et al. Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. Am J Pathol 2002;161:1743–1748.
2. Huang J, Qi R, Quackenbush J, et al. Effects of ischemia on gene expression. J Surg Res 2001;99:222–227.
3. Spruessel A, Steimann G, Jung M, et al. Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision. Biotechniques 2004;36:1030–1037.
4. Baechler EC, Batliwalla FM, Karypis G, et al. Expression levels for many genes in human peripheral blood cells are highly sensitive to *ex vivo* incubation. Genes Immun 2004;5:347–353.
5. An Analysis of Blood Processing Methods to Prepare Samples for GeneChip Expression Profiles (Technical Note). Available at http://media.affymetrix.com/support/technical/technotes/blood_technote.pdf 2003.
6. Barnes MG, Grom AA, Thompson SD, et al. Subtype-specific peripheral blood gene expression profiles in recent-onset juvenile idiopathic arthritis. Arthritis Rheum 2009;60:2102–2112.
7. Griffin TA, Barnes MG, Ilowite NT, et al. Gene expression signatures in polyarticular juvenile idiopathic arthritis demonstrate disease heterogeneity and offer a molecular classification of disease subsets. Arthritis Rheum 2009;60:2113–2123.
8. Fall N, Barnes M, Thornton S, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. Arthritis Rheum 2007;56:3793–3804.
9. Irizarry RA, Bolstad BM, Collin F, et al. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003;31:e15.
10. Bailey IR, Thurlow VR. Is suboptimal phlebotomy technique impacting on potassium results for primary care? Ann Clin Biochem 2008;45(Pt 3):266–269.
11. Boyanton BL, Jr., Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–2247.
12. Shweiki D, Itin A, Soffer D, et al. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992;359:843–845.
13. Bose D, Meric-Bernstam F, Hofstetter W, et al. Vascular endothelial growth factor targeted therapy in the perioperative setting: implications for patient care. Lancet Oncol 2010;11:373–382.
14. Xia M, Sui Z. Recent developments in CCR2 antagonists. Expert Opin Ther Pat 2009;19:295–303.
15. Emmelkamp JM, Rockstroh JK. CCR5 antagonists: comparison of efficacy, side effects, pharmacokinetics and interactions—review of the literature. Eur J Med Res 2007;12:409–417.
16. Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol 2002;2:410–416.
17. Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 2003;100:2610–2615.

Address correspondence to:
Dr. Michael G. Barnes
William S. Rowe Division of Pediatric Rheumatology
Cincinnati Children's Hospital Medical Center
3333 Burnet Ave., ML 4010
Cincinnati, OH 45229

E-mail: michael.barnes@cchmc.org

Received 29 March, 2010/Accepted 3 May, 2010

Appendix. Three Hundred Fifty-Three Probe Sets Indicating Genes with Differential Expression Between To and T4 Samples

Probe ID[a]	*Gene symbol*	*Fold change*[b]	
		2 h/0 h[c]	*4 h/0 h*[d]
204622_x_at	NR4A2	5.2877	8.9551
216248_s_at	NR4A2	5.4105	8.8413
205239_at	AREG///LOC653193	4.6343	8.1447
36711_at	MAFF	4.7111	7.0973
213933_at	PTGER3	3.2406	6.4005
219312_s_at	ZBTB10	2.7223	5.8898
202464_s_at	PFKFB3	3.266	5.8483
208078_s_at	SNF1LK	4.1568	5.8328
208937_s_at	ID1	3.9189	5.8208
203821_at	HBEGF	3.1582	5.7492
217739_s_at	PBEF1///RP11-92J19.4	2.9129	5.6252
1554309_at	EIF4G3	2.6247	5.4238
209967_s_at	CREM	3.4671	5.2409
233899_x_at	ZBTB10	2.4014	5.2317
228562_at	—	2.901	5.2283
213524_s_at	G0S2	3.772	5.1801
207630_s_at	CREM	3.5328	5.1701
224454_at	ETNK1	2.8282	5.1389
230511_at	CREM	3.7946	5.1284
204621_s_at	NR4A2	3.235	5.0914
214508_x_at	CREM	3.4216	5.049
222309_at	C6orf62	2.4626	4.8293
219228_at	ZNF331	2.6557	4.6216
218880_at	FOSL2	2.8545	4.4736
222180_at	YES1	2.5314	4.4144
240038_at	ELL2	2.6019	4.3908
202861_at	PER1	2.6954	4.3866
201466_s_at	JUN	1.781	4.2976
204141_at	TUBB2A	2.7917	4.231
202859_x_at	IL8	2.5225	4.0768
241740_at	CREM	3.5468	3.9794
227613_at	ZNF331	2.4938	3.8653
1552908_at	C1orf150	2.6113	3.7847
38037_at	HBEGF	2.1529	3.771
225262_at	FOSL2	2.8867	3.7097
201464_x_at	JUN	1.9194	3.6948
205548_s_at	BTG3	2.2915	3.6902
236495_at	PBEF1	1.6075	3.6901
233952_s_at	ZNF295	1.9552	3.5791
1562255_at	SYTL3	2.335	3.5712
1559975_at	BTG1	2.4178	3.5277
210512_s_at	VEGF	1.8886	3.4847
204440_at	CD83	2.1959	3.4748
228062_at	NAP1L5	2.3574	3.4686
230133_at	MNAB	2.5067	3.4311
203543_s_at	KLF9	2.1823	3.4159
222044_at	—	2.2031	3.3953
1554036_at	ZBTB24	2.772	3.3849
225884_s_at	ZNF336	2.2638	3.3824
204286_s_at	PMAIP1	1.8584	3.3814
242712_x_at	LOC653086///LOC653489///LOC653596	1.8887	3.3689
1556361_s_at	ANKRD13C	1.8804	3.3257
1568665_at	RNF103	2.2975	3.3193
213134_x_at	BTG3	2.2166	3.3135
1557257_at	BCL10	2.1335	3.3121
217738_at	PBEF1	2.0389	3.3118
1569136_at	MGAT4A	2.4002	3.3043
231182_at	WASPIP	2.2977	3.2973
242243_at	TMF1	1.5683	3.2954
222815_at	RNF12	1.7508	3.2557
211506_s_at	IL8	2.2651	3.2343

(*continued*)

Appendix. (Continued)

Probe ID[a]	Gene symbol	Fold change[b]	
		2 h/0 h[c]	4 h/0 h[d]
60084_at	CYLD	1.862	3.2292
210837_s_at	PDE4D	1.9635	3.2114
202643_s_at	TNFAIP3	2.5643	3.2094
241985_at	JMY	1.8644	3.2086
1555476_at	IREB2	2.5553	3.2083
211458_s_at	GABARAPL1///GABARAPL3	2.2323	3.1766
203574_at	NFIL3	2.0392	3.1158
219622_at	RAB20	1.7122	3.0976
1569263_at	SLC16A3	2.0431	3.0744
218319_at	PELI1	1.9741	3.0721
208869_s_at	GABARAPL1	1.8508	3.0551
242903_at	IFNGR1	1.5903	3.044
243664_at	TXNL1	1.6132	3.0196
232044_at	RBBP6	2.0252	3.0159
225539_at	ZNF295	1.8582	3.0102
219094_at	ARMC8	1.8875	3.001
226370_at	KLHL15	2.0354	2.997
202672_s_at	ATF3	1.4894	2.9967
233127_at	ZNF331	1.7927	2.9944
221986_s_at	KLHL24	1.8226	2.966
1555167_s_at	PBEF1	2.0741	2.9628
1554306_at	ITPKB	2.1793	2.9596
228063_s_at	NAP1L5	2.3632	2.959
243857_at	MORF4L2	1.3143	2.9586
229718_at	CG018	2.0091	2.949
238796_at	YTHDC1	1.9338	2.9354
233309_at	TMEM2	2.0113	2.9252
203372_s_at	SOCS2	2.0505	2.9183
202558_s_at	STCH	1.7833	2.8712
235592_at	ELL2	1.6639	2.8409
1553134_s_at	C9orf72	1.7996	2.8148
202932_at	YES1	1.6396	2.8141
205214_at	STK17B	2.1198	2.7996
1555963_x_at	B3GNT7	1.9141	2.7986
204285_s_at	PMAIP1	1.6905	2.795
1553861_at	TCP11L2	1.8632	2.7942
231990_at	USP15	1.945	2.7884
202644_s_at	TNFAIP3	2.3037	2.7877
211924_s_at	PLAUR	1.6585	2.7822
226608_at	LOC388272	1.8214	2.7687
213281_at	JUN	1.3926	2.7683
242176_at	MEF2A	1.8762	2.7647
221563_at	DUSP10	1.4504	2.7587
202499_s_at	SLC2A3	1.9353	2.7522
1556239_a_at	HERPUD2	2.0926	2.7461
205281_s_at	PIGA	1.7789	2.738
228846_at	MXD1	1.838	2.7347
209545_s_at	RIPK2	1.763	2.7331
210845_s_at	PLAUR	1.7088	2.7267
228181_at	SLC30A1	1.4527	2.7179
1554037_a_at	ZBTB24	2.2592	2.7114
225955_at	MED25///METRNL///LOC653506	2.1526	2.6876
222624_s_at	ZNF639	2.203	2.6807
214696_at	MGC14376	2.0627	2.6761
226275_at	MXD1	1.7789	2.6754
209803_s_at	PHLDA2	1.3385	2.6439
218486_at	KLF11	1.8841	2.641
224352_s_at	CFL2	1.8999	2.6398
202843_at	DNAJB9	1.8769	2.6257
227309_at	YOD1	1.8195	2.6186
222142_at	CYLD	1.5623	2.6162
227718_at	PURB	1.6592	2.6145

(*continued*)

Appendix. (Continued)

Probe ID[a]	*Gene symbol*	*Fold change*[b]	
		2 h/0 h[c]	*4 h/0 h*[d]
1555274_a_at	SELI	1.6311	2.6088
225557_at	AXUD1	2.1334	2.5998
213758_at	COX4I1	2.1092	2.5914
203751_x_at	JUND	1.9964	2.5908
202988_s_at	RGS1	1.8381	2.5897
1555281_x_at	ARMC8	1.9612	2.5884
210836_x_at	PDE4D	1.9332	2.5716
204014_at	DUSP4	1.7219	2.5612
225950_at	SAMD8	1.7527	2.555
228962_at	—	2.4711	2.552
211137_s_at	ATP2C1	1.66	2.5507
209457_at	DUSP5	2.352	2.5505
209020_at	C20orf111	1.869	2.5422
230134_s_at	MNAB	1.9244	2.5405
202241_at	TRIB1	1.5014	2.539
230802_at	—	1.8712	2.5229
207361_at	HBP1	1.972	2.5225
202393_s_at	KLF10	1.7447	2.5212
1555962_at	B3GNT7	2.053	2.5179
218009_s_at	PRC1	1.7305	2.5112
203373_at	SOCS2	1.5295	2.5006
222088_s_at	SLC2A3	2.0713	2.4999
202498_s_at	SLC2A3	1.9717	2.499
1553133_at	C9orf72	1.7513	2.4989
234993_at	ABHD13	1.8074	2.4978
238389_s_at	—	1.6874	2.4961
204958_at	PLK3	2.0159	2.4923
224739_at	PIM3	1.8682	2.4803
1557285_at	LOC653193	1.513	2.4758
211302_s_at	PDE4B	2.606	2.4689
242669_at	UFM1	1.557	2.4684
224657_at	ERRFI1	2.1992	2.4629
223584_s_at	KBTBD2	1.7845	2.4509
232576_at	—	1.9155	2.4416
239143_x_at	RNF138	1.652	2.4353
1552644_a_at	PHC3	1.8204	2.4195
235780_at	PRKACB	1.7088	2.4114
201465_s_at	JUN	1.3337	2.4088
206374_at	DUSP8	1.7425	2.4062
218311_at	MAP4K3	1.5526	2.4006
226811_at	FAM46C	2.115	2.3995
1554549_a_at	WDR20	1.9178	2.3993
211423_s_at	SC5DL	1.8531	2.3989
1557459_at	SNF1LK2	1.5047	2.395
216834_at	RGS1	1.7235	2.3864
213805_at	ABHD5	1.4863	2.3721
201195_s_at	SLC7A5	1.6834	2.372
37028_at	PPP1R15A	1.4221	2.3542
203542_s_at	KLF9	1.4991	2.3531
212665_at	TIPARP	1.8848	2.346
243463_s_at	RIT1	1.3348	2.3438
229899_s_at	HSUP1	1.8462	2.3411
215501_s_at	DUSP10	1.484	2.3342
224797_at	ARRDC3	1.8174	2.3328
201745_at	PTK9	1.8115	2.3255
224453_s_at	ETNK1	1.4933	2.3238
215889_at	SKIL	1.3378	2.3207
230170_at	OSM	2.265	2.2923
226345_at	—	1.67	2.2914
236223_s_at	RIT1	1.1963	2.2896
234907_x_at	POLB	1.6498	2.2841
200731_s_at	PTP4A1	1.811	2.2823

(*continued*)

Appendix. (Continued)

Probe ID[a]	Gene symbol	Fold change[b]	
		2 h/0 h[c]	4 h/0 h[d]
202497_x_at	SLC2A3	2	2.2822
216236_s_at	SLC2A3	2.0204	2.2787
214326_x_at	JUND	1.697	2.2744
202014_at	PPP1R15A	1.4726	2.2677
204908_s_at	BCL3	1.824	2.2656
206877_at	MXD1	1.9677	2.2589
201751_at	JOSD1	1.8399	2.2569
214007_s_at	PTK9	2.2865	2.2558
209383_at	DDIT3	1.4827	2.2509
219624_at	BAG4	1.4673	2.2485
1560739_a_at	UBE3C	1.6187	2.2419
225283_at	ARRDC4	1.5707	2.2404
209694_at	PTS	1.7733	2.2386
225954_s_at	MIDN	1.4918	2.2373
209795_at	CD69	1.5999	2.2349
238035_at	SP3	1.7086	2.2321
225699_at	C7orf40	1.691	2.231
206919_at	ELK4	1.38	2.2309
209211_at	KLF5	1.6051	2.2256
208868_s_at	GABARAPL1	1.7702	2.2253
223746_at	STK4	1.3786	2.2227
204299_at	FUSIP1///LOC642558	1.4299	2.2204
202657_s_at	SERTAD2	1.5966	2.2196
244103_at	C1orf55	1.4718	2.2185
243371_at	—	1.2476	2.217
219382_at	SERTAD3	1.5925	2.2096
1559582_at	RHOQ	1.634	2.2063
227979_at	RBM4B	1.6985	2.2013
227521_at	FBXO33	2.0717	2.2007
205241_at	SCO2	1.6203	2.1977
205409_at	FOSL2	1.9516	2.1974
201170_s_at	BHLHB2	1.5799	2.1973
230380_at	THAP2	1.646	2.1963
226732_at	RBM33	1.6297	2.1953
36829_at	PER1	1.5855	2.1948
220330_s_at	SAMSN1	1.4587	2.1929
1555279_at	ARMC8	1.5154	2.1899
1559121_s_at	ARIH2	1.7287	2.1812
227680_at	ZNF326	1.556	2.1762
200733_s_at	PTP4A1	1.481	2.1759
209185_s_at	IRS2	1.8139	2.1745
241018_at	TMEM59	1.7747	2.1732
213538_at	SON	1.7143	2.1713
201502_s_at	NFKBIA	1.8469	2.1713
1556750_at	LOC153577	1.4175	2.1679
1554469_at	BTBD15	1.355	2.1672
212373_at	FEM1B	1.7173	2.1652
203659_s_at	RFP2	1.6565	2.1647
223527_s_at	CDADC1	1.5857	2.1636
229955_at	FBXO3	1.553	2.1617
218881_s_at	FOSL2	1.6893	2.1598
226650_at	ZFAND2A	1.7917	2.1594
230304_at	—	1.6304	2.1564
202083_s_at	SEC14L1	1.3439	2.1561
204244_s_at	DBF4	1.5495	2.1544
200730_s_at	PTP4A1	1.6072	2.1488
221727_at	—	1.6197	2.1482
235670_at	STX11	1.3933	2.1472
221919_at	—	1.5308	2.1456
209300_s_at	NECAP1	1.6498	2.1448
218940_at	C14orf138	1.6503	2.1436
1554089_s_at	SBDS///SBDSP	1.6481	2.1423

(*continued*)

Appendix. (Continued)

Probe ID[a]	Gene symbol	Fold change[b]	
		2 h/0 h[c]	4 h/0 h[d]
230748_at	SLC16A6	1.6112	2.1411
222669_s_at	SBDS	1.6738	2.14
216015_s_at	CIAS1	1.7615	2.1398
227697_at	SOCS3	1.7134	2.1357
1554571_at	APBB1IP	1.5946	2.1335
231863_at	ING3	1.582	2.1324
238719_at	—	1.471	2.1315
204491_at	PDE4D	1.8871	2.1279
218379_at	RBM7	1.5762	2.1261
202284_s_at	CDKN1A	1.6065	2.1256
220306_at	FAM46C	1.8422	2.125
226830_x_at	LOC440309	1.5305	2.1241
212240_s_at	PIK3R1	1.756	2.1235
214060_at	AMY1A///SSBP1	1.8757	2.1225
234055_s_at	ZNF336	1.5654	2.1211
1556911_at	ALMS1	1.1536	2.1195
241385_at	LARP7	1.4494	2.119
228693_at	CCDC50	1.4426	2.1181
238488_at	IPO11	1.2186	2.1138
226970_at	FBXO33	1.6576	2.1127
222808_at	GLT28D1	1.4785	2.1123
244219_at	WTAP	1.7084	2.1063
200989_at	HIF1A	1.4106	2.1018
211998_at	H3F3B	1.8898	2.1008
218708_at	NXT1	1.6595	2.0998
242975_s_at	GNAS	1.2272	2.0946
226206_at	MAFK	1.7976	2.0904
41577_at	PPP1R16B	1.5225	2.0887
1554929_at	KIAA0999	1.5522	2.0872
212666_at	SMURF1	1.3985	2.0866
228468_at	MASTL	1.3022	2.085
218810_at	ZC3H12A	1.7462	2.082
222018_at	NACA///NACAP1///LOC389240	1.6582	2.0819
213537_at	HLA-DPA1	1.6066	2.0813
224663_s_at	CFL2	2.0207	2.081
226390_at	STARD4	1.5828	2.0764
227577_at	EXOC8	1.6527	2.0717
200664_s_at	DNAJB1	1.8485	2.0689
204799_at	ZBED4	1.6269	2.0675
238633_at	EPC1	1.6999	2.0655
228180_at	SMU1	1.6351	2.0634
201169_s_at	BHLHB2	1.7168	2.0625
209345_s_at	PI4KII	1.5147	2.0621
239494_at	LOC646725///LOC649431	1.6368	2.0608
238455_at	—	1.6264	2.0598
201341_at	ENC1	1.9072	2.0533
204370_at	HEAB	1.7027	2.0516
236196_at	—	1.5068	2.0429
200666_s_at	DNAJB1	1.6874	2.0397
212374_at	FEM1B	1.6998	2.0381
220239_at	KLHL7	1.5973	2.0372
221768_at	SFPQ	1.5509	2.0359
223598_at	RAD23B	1.6276	2.0343
203708_at	PDE4B	2.0592	2.0342
225951_s_at	LOC440309///LOC649908	1.5941	2.0342
221763_at	JMJD1C	1.3942	2.0265
242255_at	WDR37	1.2723	2.0258
205681_at	BCL2A1	1.3674	2.0221
1569864_at	SERAC1	1.3365	2.0106
1570394_at	XRN1	1.4304	2.0053
207513_s_at	ZNF189	0.5791	0.4999
212407_at	KIAA0859	0.6241	0.4969

(*continued*)

Appendix. (Continued)

Probe ID[a]	*Gene symbol*	*Fold change*[b]	
		2 h/0 h[c]	*4 h/0 h*[d]
218805_at	GIMAP5	0.6386	0.4966
223404_s_at	C1orf25	0.5826	0.4961
208893_s_at	DUSP6	0.5503	0.4955
1552316_a_at	GIMAP1	0.5609	0.4949
218979_at	RMI1	0.5514	0.4947
207907_at	TNFSF14	0.6168	0.4946
208891_at	DUSP6	0.4888	0.4937
238907_at	LOC284323	0.6243	0.4922
230226_s_at	JARID1A	0.6606	0.4913
220235_s_at	C1orf103	0.5597	0.488
228920_at	ZNF260	0.5932	0.4869
231576_at	ETNK1	0.7761	0.4838
221081_s_at	DENND2D	0.6306	0.4826
219777_at	GIMAP6	0.5575	0.48
223583_at	TNFAIP8L2	0.5994	0.4747
209828_s_at	IL16	0.6659	0.4737
206206_at	CD180	0.6085	0.4686
226230_at	SMEK2	0.618	0.4657
228190_at	CTR9	0.5411	0.4623
226481_at	VPRBP	0.5429	0.4613
235085_at	DKFZp761P0423	0.5794	0.4582
226977_at	LOC492311	0.6134	0.4544
216379_x_at	CD24	0.5512	0.4533
223751_x_at	TLR10	0.6385	0.4482
220992_s_at	C1orf25	0.5581	0.4437
224953_at	YIPF5	0.5	0.4376
219243_at	GIMAP4	0.5749	0.4371
218242_s_at	SUV420H1	0.5972	0.4344
227335_at	DIDO1	0.5739	0.4299
229367_s_at	GIMAP6	0.5808	0.427
226423_at	PAQR8	0.5985	0.4188
200799_at	HSPA1A	0.617	0.4116
206978_at	CCR2	0.5425	0.408
227626_at	PAQR8	0.5885	0.4058
240646_at	GIMAP8	0.5375	0.4037
206991_s_at	CCR5///LOC653725	0.5257	0.3864
226041_at	NAPE-PLD	0.5368	0.3817
207794_at	CCR2	0.6309	0.3811
205898_at	CX3CR1	0.5622	0.3734
222566_at	SUV420H1	0.4814	0.3719
233461_x_at	ZNF226	0.5794	0.3675
200800_s_at	HSPA1A///HSPA1B	0.6225	0.3664
235306_at	GIMAP8	0.4554	0.3547
230337_at	SOS1	0.3715	0.344

[a]Affymetrix U133 Plus 2.0 GeneChip® probe set IDs and annotations.
[b]Fold change as ratio of geometric means.
[c]Expression at 2 h/expression at 0 h.
[d]Expression at 4 h/expression at 0 h.