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Genome wide responses of murine lungs to dietary α-tocopherol

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Abstract

α-tocopherol (α-T) may affect biological processes by modulating mRNA concentrations. This study screened the responses of ~15,000 lung mRNAs to dietary α-T in mice. The lung was chosen as the target organ because it is subjected to cyclical variations in oxidant and inflammatory stressors and α-T has been implicated in their modulations. The analysis identified ~400 mRNAs sensitive to α-T status of lungs determined by dietary α-T. The female lung transcriptome appears to be more sensitive to the α-T status than that of the male lungs. Here, we focus on the induction of 13 cytoskeleton genes by dietary α-T because they were similarly induced in the male and the female lungs. Their inductions were confirmed by quantitative-real-time-polymerase chain reaction (qRT-PCR). Immunohistochemical analyses of three of the encoded proteins suggest that they are expressed in lung vasculature and alveolar regions. The data suggest that the lung α-T status may modulate cytoarchitecture of lungs.

Keywords: *Tocopherol, vitamin E, smooth muscle, oligonucleotide-arrays, microarrays, cytoskeleton*

Introduction

α-Tocopherol (α-T) is the most abundant member of vitamin E family in the mammalian tissues [1–3]. Dietary deficiency of α-T is rare but causes fetal death in rats [4], impairs the development of placenta in mice [5], decreases litter size in pigs [6] and causes neuromuscular dysfunctions in rodents, chicken, cattle and humans [1,7,8]. These are complex pathological outcomes in which deficiency of α-T may deregulate the transcription of multiple gene families. The precise mechanism(s) by which tissue α-T prevents these well-defined vitamin E deficiency symptoms remain elusive. Antioxidant mechanism of vitamin E action was suggested by early researchers who observed that it could prevent the oxidation of lard used as a source of fat in semi-synthetic diets for rodents (reviewed in Ref. [9]). Subsequent studies identified α-T as the active chemical that acted as an antioxidant in biological membranes [10–12]. The antioxidant function of α-T *in vivo* became popular after a series of publications following the initial description of the inhibition of hematin-catalyzed oxidations by α-T [13] and was further supported by many subsequent studies [14]. The antioxidant properties of α-T have been hypothesized to offer protection against pathologies associated with increased oxidant stress [15,16]. However, outcomes of clinical trials based on the antioxidant hypothesis of α-T have shown minimal or insignificant effects and have generated controversy [17–19] suggesting the need for better understanding of the actions of α-T *in vivo* [20,21].

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α-T has been suggested to affect the expression of five groups of genes through non-antioxidant mechanisms [22]. Genome-wide mRNA analysis of tissues from rats and mice fed different concentrations of α-T suggest tissue specific effects of this vitamin on mRNA concentrations which may primarily be regulated by gene transcription. For example, α-T affected the expression distinct sets of genes in liver [23] compared to those in the testes and the adrenal glands [24] or the hippocampus [25]. Similarly, chronic α-T deficiency imposed at birth in mice engineered to silence the expression of α-T transfer protein resulted in the deregulation of multiple genes in the cerebral cortex [26] and in other organs [27] of these transgenic mice. The latter studies prompted the hypothesis that α-T may act by affecting the activity of retinoic-acid-related-orphan receptor-α (ROR-α), a transcription factor in neuronal and other tissues. ROR-α is a member of nuclear receptor superfamily that includes pregnane-X-receptor which has also been implicated in the catabolism of the vitamin E family [28].

The present study has focused on the *in vivo* sensitivity of the genome of a vital organ, the lung to α-T. The lung is subjected to dynamic and cyclical fluctuations in oxidative and non-oxidative stresses from both exogenous and endogenous sources. Therefore, the lung presents an attractive organ to explore the antioxidant and non-antioxidant actions of α-T *in vivo*. Male and female C57BL6 mice were fed, for four months post weaning, diets that varied in the α-T content by two orders of magnitude. The responses of ~15,000 lung genes were studied. The results and discussion are focused on a cluster of 13 genes that share common functions and were induced by α-T in both male and female mice. The induction of these genes was independently confirmed by quantitative-real-time-polymerase chain reaction (qRT-PCR). Preliminary immunohistochemical analyses suggest that the proteins encoded by three of the cytoskeleton genes are expressed in lung vasculature and respiratory epithelium.

Materials and methods

Diets

Diets were purchased from Purina Test Diets, Richmond, PA, USA. The composition of diets (AIN-93) used in these experiments was that recommended by American Institute of Nutrition for rodents [29]. The basal α-T-poor diet was obtained by substituting the source of fats in AIN-93 diet with vitamin E-stripped oils and substituting the vitamin mix with vitamin mix lacking in α-T. The α-T-poor diet contained < 10 IU of α-T/kg of diet (Catalog # 7248). The diets containing either 35 or 1000 IU of α-T/kg diet were obtained by the addition of the appropriate amounts of dl-α-tocopheryl acetate to the α-T-poor diet. The mice were allowed to feed on the pelleted diets *ad libitum*.

Mice (C57B16)

Mice (C57Bl6) were purchased from Charles River Laboratories, Wilmington, MA, USA. Four weeks old male or female mice were randomly assigned to feed on one of the three diets: < 10 IU α-T/kg diet, the "α-T-poor" diet, or the 35 IU α-T/kg diet, "α-T-normal" diet or the 1000 IU α-T/kg diet "α-T-high" diet. Each dietary group contained 4–6 male or female mice. The mice were allowed to feed on the respective diets for four months and then euthanized with an overdose of pentobarbital (> 100 mg/kg body wt). Blood was collected by cardiac puncture with heparinized 1 ml syringes, subjected to centrifugation to obtain plasma which was then stored at − 70°C. Lungs were rapidly dissected free of the large airways and frozen on dry-ice followed by storage at − 70°C until further analysis.

Two additional groups of male and female mice were treated similarly to the above groups of mice. The lungs from one group were used for the confirmation of GeneChip data by qRT-PCR and those from another group were used for immunohistochemical studies.

α-T concentrations in the plasma (10–20 μl) and in the lungs (~20 mg) from the first group of mice were analyzed as described in Ref. [30].

RNA extraction

RNA extraction from the lungs was performed as previously described [31]. Approximately half a lung (~50 mg) from each mouse was homogenized in 1 ml of trizol reagent (Invitrogen, Carlsbad, CA) and a pellet of total RNA was obtained using the procedures described by the manufacturer. The total RNA pellet from each lung was dissolved in RNAase–DNAase free water to obtain a concentration of 2.5 μg of RNA/μl of solution. An equivalent aliquot of RNA (5 μg) from each lung was combined, group-wise, to obtain a total of five pooled RNA samples; a pooled RNA sample from the male mice fed the α-T-poor diet, a pooled RNA sample from the male mice fed the α-T-high diet and three pooled RNA samples from each group of the female mice fed the α-T-poor, the α-T-normal, or the α-T-high diet, respectively. Total RNA from the male mice fed the α-T-normal diet was lost during the isolation procedure. Each pooled sample was analyzed with a mouse genome 430A 2.0 GeneChip (Affymetrix, Santa Clara, CA).

GeneChip analyses

GeneChip analyses of the pooled total RNA samples were performed as previously described [26]. A 20 μg aliquot of total RNA from each pooled sample was reverse transcribed, followed by 2nd strand synthesis, cRNA amplification in the presence of biotin-labeled nucleotides and fragmentation as described in the Affymetrix One Cycle Sample Preparation protocol (Affymetrtix, Santa Clara, CA). The fragmented, biotin labeled cRNA samples were hybridized to mouse genome 430A 2.0 high density oligonucleotide arrays with 22,626 probe sets representating transcripts and variants from over 14,000 well characterized mouse genes (Affymetrix, Santa Clara, CA). The hybridizations, washings, labelings and scannings of the GeneChips were performed as described in the Affymetrix protocols by Microarray Core Facility in the UC Davis Genome and Biomedical Sciences Facility.

The GeneChip data were analyzed by GeneChip Operating System 1.3 (GCOS 1.3, Affymetrix). The upper limit of p value for statistically reliable detection of an mRNA was 0.05 (except for "batch analysis", see below), independent of its signal intensity (usually >10), because the detected mRNA can be confirmed by an independent method such as qRT-PCR. The p-value for detection of mRNAs discussed here ranged from 0.0001 to 0.05 (except for "batch analysis", see below) and the signal intensity ranged from 10 to 10,000 units.

The lists of α-T sensitive genes were obtained by using "batch analysis" function in GCOS 1.3. The data for *all* of the 22,626 probe sets from the lungs of the female mice fed the α-T-poor diet were used as baseline and compared with those from the lungs of the female mice fed the α-T-high diet. Similarly, the data for *all* of the 22,626 probe sets from the lungs of the male mice fed the α-T-poor diet were used as baseline and compared with those from the lungs of the male mice fed the α-T-high diet. The lists (in Excel format) of α-T sensitive genes were then sorted to satisfy three requirements: (a) the mRNA must be detectable with a p-value of ≤ 0.05 in at least one of the two samples being compared; (b) the fold-change be ≥ 2; and (c) the mRNA have an annotation that suggests either a known or a predicted function. Using the same criteria a list of genes sensitive to the α-T-normal diet was also generated for the female mice. The lower limit of 2-fold change was selected because our studies suggest that this selection criterion decreases the probability of obtaining "false positives" for selecting genes for further consideration. This may be particularly desirable in microarray experiments performed on pooled RNA samples, an analytical strategy that results in loss of statistical information (discussed in Results).

Validation

Validation of changes in mRNA expression by qRT-PCR.

Total RNA samples for the confirmation study were obtained from a different cohort of mice that were treated identically to those used for the GeneChip analysis. Unlike the RNA samples for GeneChip analysis, those for qRT-PCR analysis included RNA samples from the male mice fed the normal diet. The individual RNA sample from each lung was used for validation of GeneChip data and for obtaining statistical significance of the changes in the expression of the gene in the defined dietary group of mice. An aliquot equivalent to 5 μg of total RNA extracted from each lung was reverse-transcribed to obtain cDNA in a final volume of 20 μl solution consisting of buffer, oligo-dT primer, DTT, dNTPs and Superscript-II reverse transcriptase (Invitrogen, Carlsbad, CA). RT-PCR method with SYBR as fluorescent reporter was used to quantify the expression of selected genes identified by GeneChip analysis. All the gene specific primers (Table I) were designed with Primer Express 1.0 software (Applied Biosystems) using the gene sequences obtained from Affymetrix Probeset IDs. The reaction was carried out in 96 well optical plates containing 6.25 ng RNA in each well. The applied RNA quantity was normalized by amplifying cDNA samples simultaneously with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) specific primers. The transcript levels were measured by RT-PCR using the ABI PRISM 7700 Sequence detection system (PE Applied Biosystems, Foster City, CA). The PCR amplification parameters were: initial denaturation step at 95°C for 10 min followed by 40 cycles, each at 95°C for 15 s (melting) and 60°C for 1 min (annealing and extension). The $2^{-}\Delta\Delta CT$ method [32] was used to calculate relative changes in gene expression determined from qRT-PCR experiments (Applied Biosystems User Bulletin No.2 (P/N4303859)). The threshold cycle, C_t, which correlates inversely with the target mRNA levels, was measured as the cycle number at which the SYBR Green emission increases above a preset threshold level. The specific mRNA transcripts were expressed as fold difference in the expression of the specific mRNAs in RNA samples from the lungs of mice fed the α-T-poor diet compared to those in the lungs of mice fed either the α-T-normal diet or the α-T -high diet.

Immunohistochemical

Immunohistochemical procedures for the identification of cellular localization of the specific proteins were essentially as described in Ref. [33]. Briefly, lungs were fixed by intratracheal instillation of paraformaldehyde (4% in phosphate buffered saline, pH 7.5) at a constant pressure of 20 cm H_2O for 1 h. The trachea were then ligated and the lungs placed in the fixative overnight. The fixed tissues were sliced, dehydrated and embedded in paraffin. Adjacent

Table I. List of oligonucleotide primers for qRT-PCR.

Gene name	Forward primer	Reverse primer
Actin, alpha 1, skeletal muscle	ACGCTCTTCCAGCCTTCCTT	GACATGACGTTGTTGGCATACAG
Myosin light chain	CCCAGAAGGGAAGGGCAC	TGGGAAAATCGGTCGCAC
Myosin, heavy polypeptide 1	GAAGAGTGATTGATCCAAGTGCAG	AAAGTTATGAGTACAAAACAGAGTGACAAAG
Myosin, heavy polypeptide 8	GAAGCATGAAAGAAGAGTAAAGGAACTTA	CGCCTGTAATTTGTCCACCAG
Myosin, light polypeptide 3	AGAGGACTCCAACGGCTGC	CTTGGCCTGCCCTAGGCT
Tropomyosin 2, beta	GAGACCCGAGCAGAGTTTGC	GGTTTTCTCCAACTTTGCCACA
Troponin C2, fast	ACAGGAACGCAGACGGCTAC	AAGCCCGGAAAATCTCAGCTA
Troponin I, skeletal, fast 2	GAAGAGGAGAAATATGACATGGAGGT	CTCCTTGCTGCTCTTCTGCAC
Keratin complex 1, acidic, gene 13	GAAAGTACGTTGGCAGAGACAGAGT	TGCTGCTGATGAGCCCCT
Keratin complex 1, acidic, gene 15	TGGAGGCTCAGAACCAGGAG	GCCGAGTCTTGATGTCCAGC
Keratin complex 2, basic, gene 4	TCTTCCGCCACCATCACC	CTCAGGGCCATCAGGATCC
Loricrin	CTGGCCGTGCAAGTAAGGTC	TCCAGCTCTGTTGTCTCCGTT
Repetin	ATTTTGGACTTTGGTGAGCATTG	GCTTCTCACTGTTTCATCCAAGTCTA

The nucleotide sequence (5′–3′) for each primer was obtained using Primer Express software as described in the Methods. Each primer was custom prepared by Qiagen, Valencia, CA.

sections were stained with hematoxylin and eosin or with the specific antibody. The latter were lightly counter stained with hematoxylin (to stain the nuclei) and the processed sections were visualized by standard light microscopy. Mouse monoclonal antibodies to muscle specific actin (VP-M659) and myosin heavy chain (VP-M667) were purchased from Vector Laboratories, CA. Mouse monoclonal antibody to tropomyosin [CH1] was purchased from Abcam, Cambridge, MA. The digital images were obtained with Zeiss-Axiovision system.

Statistical data

Statistical data for plasma and lung α-T concentrations, and for fold-changes in gene expression obtained by qRT-PCR were analyzed by GraphPad PRISM (version 4.0; GraphPad Software, San Diego, CA). The data are reported as means ± SEMs. An unpaired Student's *t*-test or two-way analysis of variance with repeated measures was used as appropriate for comparisons between the treatments. All data were considered statistically significant when *P*-values were ≤ 0.05.

Results and discussion

Dietary content of α-T determines the α-T concentrations in plasma and lungs

At the end of four months of dietary treatments, the whole body weights within each gender group were not significantly different between the mice fed the different diets (Figure 1). These data suggest a lack of any obvious adverse dietary effect that may be caused by altered food consumption due to poor dietary α-T concentrations or due to pharmacological concentrations of α-T in the diets over the four month period.

The plasma α-T concentrations were low in the mice fed the α-T-poor diet and there were no significant differences between the male and the female plasma α-T concentrations (Figure 2). As expected, mice fed the α-T-normal or the α-T-high diets showed significantly higher plasma α-T concentrations compared to plasma α-T concentrations of mice fed the α-T-poor diet (Figure 2). The concentration of plasma α-T in mice fed the α-T normal diet was higher than that previously reported in normal mice fed a similar diet [34] and similar to that reported in a previous study [35]. The plasma α-T concentrations of the male mice were significantly higher than those of the female mice. These observations suggest gender specific differences in the regulation of circulating α-T concentrations. The plasma concentrations of α-T are determined by multiple factors that include the fat composition of the diet, supplementary concentrations of tocopherols, durations of the feeding period and the expression of receptors that transport lipoproteins across the various cell types [36–40]. Gender specific differences in the expression

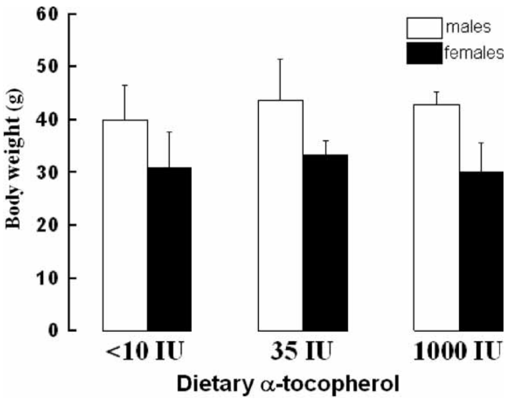
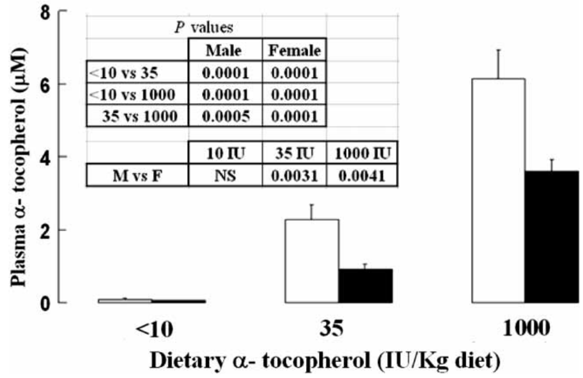


Figure 1. Body weight of mice fed the three assigned diets. The data show mean ± SEM for each group.



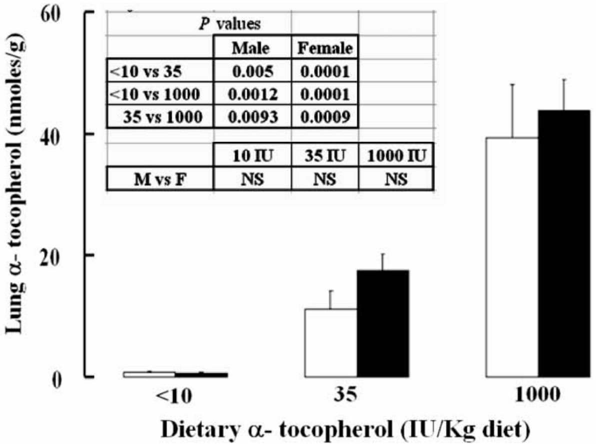
P values	Male	Female
<10 vs 35	0.0001	0.0001
<10 vs 1000	0.0001	0.0001
35 vs 1000	0.0005	0.0001

	10 IU	35 IU	1000 IU
M vs F	NS	0.0031	0.0041

Figure 2. Concentration of α-T in plasma of mice fed the three assigned diets for four months post weaning. The data show mean ± SEM of α-T concentration in the plasma from each group of mice. The inset table shows statistical analysis of the defined comparisons.

of one or more of these variables may contribute towards the observed gender specific differences in the plasma concentrations of α-T detected in our study. Various studies suggest that some members of cytochrome P450 superfamily of genes affect tocopherol metabolism [41–43]. A recent study showed that the expression of CYP3A9 was 28-fold higher in the livers of female rats compared to those of the male rats [44]. In addition, CYP3A9 expression was modulated by estrogen. Although the role of CYP3A9 in the metabolism α-T remains to be studied, we speculate that such gender specific differences in the expression of α-T metabolizing enzymes may contribute to the data shown in Figure 2.

α-T concentrations in the lungs were low in the mice fed the α-T-poor diet and the gender of the mice had no effect on this parameter (Figure 3). Lungs of mice fed the α-T-normal or the α-T-high diets contained α-T concentrations that were at least 10-fold or 40-fold higher than those of the mice fed the α-T-poor diet, respectively. Unlike the significant differences in plasma α-T concentrations between male and female mice, there was no statistically significant difference in lung α-T concentrations between the two genders. Thus, any differences that might have existed between the male and the female mice in feed intake, the plasma concentrations of α-T or the metabolism of α-T did not appear to affect the steady-state concentrations of α-T in the lungs of the male and the female mice. These latter observations are noteworthy because the differences that were observed in the lung gene expression profiles between male and female mice (Table III and Appendix I and II) were unlikely to be ascribed to the α-T content of the lungs. More importantly, as discussed below, the responses of the genes encoding the cytoskeleton protein family to the lung α-T status is conserved in the male and the female mice.



P values	Male	Female
<10 vs 35	0.005	0.0001
<10 vs 1000	0.0012	0.0001
35 vs 1000	0.0093	0.0009

	10 IU	35 IU	1000 IU
M vs F	NS	NS	NS

Figure 3. Concentration of α-T in lungs of mice fed the three assigned diets for four months post weaning. The data show mean ± SEM of α-T concentration in the lungs from each group of mice. The inset table shows statistical analysis of the defined comparisons.

Lung genomic responses associated with increases in lung α-T concentrations

Current strategies for microarray analysis require independent validations of selected, differentially expressed genes irrespective of the initial analytical design. Two analytical options may be considered for microarray experiments. The preferred option involves the use of one GeneChip per RNA sample. However, the cost for such an experimental design may be formidable. An alternative experimental design, which was used in this study is pooling maximum number of RNA samples ($N \geq 4$) from each treatment group and analyzing the pooled RNA sample/GeneChip. A major disadvantage of the pooling strategy is loss of statistical information from each individual sample. This disadvantage is rectified and the statistical information is recovered by performing independent analysis of the candidate differentially expressed mRNA (identified by GeneChip analysis of pooled sample) by qRT-PCR of the mRNA in each individual sample in the treatment group, an analytical step also mandatory for the "expensive" experimental design.

Table II gives an appreciation of the reproducibility of the GeneChip assay. As noted above, five individual GeneChips were used for the five pooled RNA samples. The expression of ~15,000 mRNAs were reliably ($p < 0.05$) detected in the lungs from each group of mice; the coefficient of variation (CV) for the total number of genes detected in the five different biological samples was 2.9%. To monitor the performance of GeneChip analysis, hybridization-controls (spike-controls) are added to every sample subjected to GeneChip analysis. Table II shows signal intensities for the low and high hybridization-controls; the CVs for these control standards were 13.3 and

Table II. Reproducibility of the GeneChip assay.

Diet	<10 IU		35 IU		1000 IU				
Mice	Female	Male	Female	Male	Female	Male			
Gene Chip #	1	2	3	ND	4	5	Group mean	Group SEM	CV (%)
Average noise	3.75	3.67	4.48	ND	3.97	4.61	4.1	0.4	10.4
3′/5′ ratio actin	1.7	1.55	2.05	ND	1.44	1.91	1.7	0.3	14.5
3′/5′ ratio GAPDH	0.92	0.98	0.96	ND	1.03	0.86	1.0	0.1	6.7
Total # of genes	14,157	15,317	14,485	ND	14,698	14,727	14,677	212	2.9
Spike control (low)	145	130	102	ND	107	120	120	8	13.3
Spike control (high)	3480	3610	3390	ND	3450	3190	3420	80	4.7
Clara cell 10	10,400	10,200	9240	ND	8920	9920	9740	320	6.5
Surfactant protein C	8990	9120	7830	ND	7940	9040	8580	320	7.4
Epithelilal memp 2	3100	5080	3530	ND	3530	4900	4030	450	22.3
GP38	1880	1040	640	ND	920	1026	1100	230	41.8
SRF binding protein	25	50	50	ND	25	40	40	6	30.3

Each dietary group included 4–6 mice. Total RNA was isolated from approximately half of lung tissue from each mouse. The RNA samples from male mice fed the 35 IU diet were lost during isolation (ND). About 10 μg of total RNA from each lung was pooled, group-wise and an aliquot (20 μg) of the pooled sample was used for GeneChip (430A 2.0) analysis. The data in the table show signal intensities for two hybridization controls (spike control) and 5 of the $\sim$15,000 genes that were detected with the detection $p < 0.05$ (range 0.0001–0.05). The two hybridization controls were aliquoted from the **same sample** and added to five different pooled RNA samples for GeneChip analysis. The CV for the two controls ranged from 4.7–13.3% and indicate overall variation in the analytical procedure. The data in the table also show the expression of three genes that are considered as markers of Clara cells (Clara cell 10, CC10), type II pneumocytes (surfactant protein C, SPC) and type I pneumocyte (GP38) in lungs. The CV for the expression of CC10 and SPC for the five treatment groups were in the range of the spike controls. The expression of epithelial membrane protein 2 and SRF binding protein have not previously been described in the lungs and showed a larger CV in their detection but still considerably less the 200%, the lower limit for considering a gene as differentially and significantly affected by dietary α-T concentration. The intensity data also illustrate the large dynamic range for the detection of mRNAs by the GeneChip assay. The total number of genes detected in the five lung samples was very similar to each other (CV = 2.9).

4.7%, respectively. In addition, Table II shows the magnitude of expression for three genes that are commonly used as markers for lung specific cells; these are Clara cell specific Clara cell 10, type-II pneumocyte specific surfactant protein C and type-I pneumocyte specific glycoprotein (GP) 38. The CVs for the expression of these genes were between 6.5–41.8% (Table II). The expression of two additional genes is included in Table II. The expression of epithelial membrane protein has not previously been described in murine lungs and its expression is higher than that of GP38. Serum response factor (SRF) binding protein was chosen to illustrate that even if the signal intensity is very low compared to that of CC10, the mRNA was reliably ($p < 0.05$) detected with a CV that was similar to that of more abundantly expressed lung genes. Most of the $\sim$15,000 genes detected by the GeneChip assay showed CVs which are comparable to the CV for the detection of lung α-T concentrations (Figure 3). The significance of the latter comparison is that the CV of GeneChip assay is similar to that of the routinely used multi-step method for the detection of α-T concentrations. In addition, for all the genes shown in Table II and for most of the $\sim$15,000 genes detected by the GeneChip assay, the CV was $\ll$ 200%, the lower cut-off for calling a gene sensitive to dietary α-T.

Table III shows a summary of the total number of genes affected in the lungs of the female and the male mice fed the three different diets. The data lack statistical support and make these data just a qualitative overview of the possible effects of the gender and the lung α-T status on gene expression. The total number of genes affected by the two concentrations of α-T in the lungs of the female mice was similar, but the number of the induced or the repressed genes appears to be dose dependent. In addition, the total number of genes affected in the lungs of male mice fed the α-T-high diet appeared to be $\sim$20% of those affected in the lungs of the female mice fed the same diet. Appendix I lists all the genes that were sensitive to α-T-high diet in female lungs. Appendix II lists all the genes that were sensitive to α-T-high diet in male lungs. Appendix III lists all the genes that were sensitive to α-T-normal diet in female mice.

The gender specific differences in the sensitivity of the lung genome to the α-T status need to be evaluated by a more statistically robust experimental design. As noted above, the expression of CYP3A9 is 28-fold higher in livers of female mice compared to

Table III. Number of genes differentially affected by dietary α-tocopherol. The data were obtained by "batch analysis" as described in the Methods. The expression data from the lungs of mice fed the α-T-poor diet were used as baseline.

		Number of genes		
Sex	α-T (IU/kg)	Total	Up	Down
Females	35	405	388	17
Females	1000	486	81	405
Males	1000	84	78	6

that of the male mice and its expression is regulated by estrogen [44]. Review of the literature suggests gender related differences in lung development and susceptibility to lung diseases in humans [45–47]. Our data may hint at possible differences between the female and the male lung genomes to the overall concentrations of lung α-T (or its metabolized or oxidized products).

Effects of lung α-T status on the expression of the "classical" antioxidant genes and on CD36

A large number of investigations have postulated that α-T acts as an antioxidant in biological membranes [48–50]. Therefore, low amounts of α-T, such as that obtained in the mice fed the α-T-poor diet for four months (Figures 2 and 3), was expected to "activate" an antioxidant response that includes transcriptional activation of genes that encode antioxidant enzymes. Messenger RNA expression profiles from the five groups of mice were mined for the expression of catalase, heme-oxygenase, glutamate-cysteine ligase and superoxide dismutase genes (Table IV). The expressions of SOD1 (Table I and Appendix I), SOD2 and SOD3 (Appendix I) were high ($>$2-fold) only in the lungs of female mice fed the α-T-poor diet compared to their expression in the lungs of mice fed the α-T-high diet. However, the induction of SOD1 in the lungs of mice fed the α-T-poor diet difference was not confirmed by qRT-PCR, suggesting a false positive result from the GeneChip assay (qRT-PCR of SOD2 and SOD3 were not done). Additional qRT-PCR analysis are also necessary to exclude the possibility that the lack of change in the expression of other antioxidant genes is not a false negative result from the GeneChip assay. Our previous gene profiling studies of cells in culture showed a robust induction of glutamate-cysteine ligase (GCL) and hemeoxygenase 1 (HO-1) associated with increase in intracellular reactive oxygen species [51]. The present *in vivo* studies suggest that very low α-T concentrations in the lungs of mice fed the α-T-poor diet for four months did not generate sufficient oxidant stress to activate the transcriptional response of HO-1 and GCL genes.

The GeneChip data were also mined for the expression of CD36 which has previously been shown to be down-regulated by α-T in human aortic smooth muscle cells [52], isolated monocyte-derived macrophages [53,54], HepG2 cells [23]and in rat livers *in vivo* [23,55]. Data in Table IV show that CD36 is abundantly expressed in the lung tissue from the male and the female mice and its expression is unaffected by the α-T status of the lung. Our observations suggest that the α-T sensitivity of CD36 expression may be tissue specific. Alternatively, the discrepancy between our data in lungs and those in the livers for CD36 expression may also be explained by differences in the composition of the rodent diet, plasma concentrations of α-T and by the duration of the dietary treatment. For example, the plasma concentrations of α-T in rats were ~30 μM after ~9 months on the α-T supplemented diet [55]. This is in contrast to ~7 μM of plasma α-T after four months on the α-T-high diet. In addition to the effects of these variables the expression of CD36 may further be regulated by oxidant stress such as that presented by oxidized lipoproteins [16,54]. A limitation of the current experiments is that we did not specifically investigate mouse lung tissues for biomarkers of protein, lipid or nucleotide oxidative products.

Table IV. The expression of "classical" antioxidant and CD36 genes in the lungs from mice fed the three assigned α-T diets.

		Dietary α-T (IU/kg)				
		<10	35	1000	10	1000
		Females			Males	
Gene name	Gene symbol	GeneChip signal intensity				
Catalase	Cat	578	936	820	1341	1259
Glutamate-cysteine ligase, catalytic subunit	Gclc	652	690	535	767	872
Glutamate-cysteine ligase, modifier subunit	Gclm	159	190	234	277	299
Heme oxygenase (decycling) 1	Hmox1	80	72	58	176	138
Heme oxygenase (decycling) 2	Hmox2	254	393	366	597	496
SOD1, soluble	Sod1	3546	841	1418	2535	1974
SOD2, mitochondrial	Sod2	409	743	838	1018	899
SOD3, extracellular	Sod3	849	751	715	1208	1168
CD 36 antigen	CD36	2600	2743	2676	4525	4433
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	1797	1502	1771	2607	2593

GeneChip data from the lungs of mice were mined for the expression of genes listed in the table. The data show signal intensity for each gene in each group of mice. The expression of house keeping gene, GAPDH is shown for reference. All the listed genes except that for SOD1 were within a 2-fold range and were considered to be unaffected by the α-T status. The induction of SOD1 was not confirmed by qRT-PCR.

Cluster of genes encoding cytoskeleton and intermediate filament proteins were induced by dietary α-T

A novel contribution of this study is the identification of 13 genes that are coordinately induced by high concentrations of α-T in plasma and in the lungs from both female and male mice. In contrast to the apparent lack of any effect of the α-T status on the expression of the antioxidant genes and on most of the ~15,000 genes detected by the GeneChip assay, the induction of a cluster of 13 genes (Table V) was remarkable and became the focus of further experimentation for three reasons: (1) they were annotated to encode contractile-cytoskeleton functions and therefore are functionally related; (2) they were all induced, and therefore may share common transcription factors; and (3) their apparent co-regulated induction by α-T-high diet was detected in both female and male lung tissues and therefore likely to be a valid result.

Table V shows the data from GeneChip analysis and qRT-PCR validations. The GeneChip data show fold-change for each gene in the lungs of mice fed the α-T-high diet computed as described in the Methods; the expression data from the lungs of the mice fed the α-T-poor diet were used as baseline and those fed the α-T-high diet as test data. The GeneChip data in Table V also includes data from comparison of the female mice fed the α-T-normal diet with those fed the α-T-poor diet to determine if the induction could be seen at the normal dietary α-T concentrations. The GeneChip data suggest that the inductions of only 2 of the 13 genes were seen in the lung tissue of mice fed the α-T-normal diet. Table V also includes data from male mice fed the α-T-high diet; 11 of the 13 genes induced in the female lungs were also induced in the male lungs.

The expression of each α-T sensitive gene detected by the GeneChip assay was further verified by qRT-PCR analysis using gene specific oligonucleotide primers (Table I). About 12 of the 13 genes whose expression was identified to be increased by *α-T-high diet* in the GeneChip assay was confirmed to be induced by the qRT-PCR assay, providing a strong support for the utility of the GeneChip assay to identify differentially expressed genes with the "sample pooling" strategy described in the Methods. The data in Table V are further strengthened by the fact that they originate from two different cohorts of mice subjected to identical dietary α-T protocols on separate occasions.

Although there was good qualitative agreement between the two assays, quantitative agreement was poor. This could be caused by very different methods of amplification of the cDNA templates and the method of detection of the amplified products. In the GeneChip assay, cRNA is amplified from double stranded DNA using T7-DNA dependent RNA polymerase. The amplification takes place in the presence of biotin labeled ribose nucleotides. Following the hybridization of the biotin labeled cRNAs to the complimentary deoxy-oligonucleotides on GeneChips they are detected by fluorescent labeled avidin. In contrast, qRT-PCR assay amplifies selected DNA fragments from cDNA templates with thermostable DNA polymerase and sequence specific primers.

Table V. Confirmation of GeneChip data by qRT-PCR.

		Fold change						
		GeneChip data			qRT-PCR data			
		Females		Males	Females		Males	
Gene name	Gene symbol	35 IU	1000 IU	1000 IU	35 IU	1000 IU	35 IU	1000 IU
Tropomyosin 2, beta	Tpm2	1.4	2.5	1.7	1.6*	1.5*	1	1.7*
Myosin, heavy polypeptide 1	Myh1	1.0	3.7	19.7	15.2*	22.5*	7.5***	30.5***
Myosin, heavy polypeptide 8	Myh8	1.0	4.9	147.0	148.3*	324.9*	1.5***	39.2***
Troponin I, skeletal, fast 2	Tnni2	1.0	5.7	6.5	2.5*	6.3*	1	2.3
Myosin light chain	Mylpf	1.2	6.5	4.9	1.9*	5.5*	2.0***	4.0***
Actin, alpha 1, skeletal muscle	Acta1	1.0	8.0	19.7	3.9**	14.5**	1.9***	10.0***
Myosin, light polypeptide 3	Myl3	1.5	8.6	7.5	2.5*	4.0*	1.45*	5.8*
Keratin complex 1, acidic, gene 15	Krt1-15	1.7	10.6	1.2	1.5	2.5	1.4	1.7
Troponin C2, fast	Tnnc2	1.0	24.3	59.7	6.8*	44.5*	1.3**	5.3**
Keratin complex 2, basic, gene 4	Krt2-4	1.0	48.5	168.9	1.2	4.9*	1.4***	14.9***
Repetin	Rptn	12.1	64.0	3.5	2.3*	2.7*	1.2	1.1
Keratin complex 1, acidic, gene 13	Krt1-13	6.1	256.0	222.9	19.5*	35.9*	6.8***	17.7***
Loricrin	Lor	1.0	337.8	137.2	6.2*	15.5*	4.2***	37.4***

The table shows fold-change for each gene obtained by either the GeneChip assay or the qRT-PCR assay. The fold-change for each gene was obtained by comparing its expression in the lungs form mice fed either the α-T-normal or the α-T-high diet with that in the lungs from mice fed the α-T-poor diet.The GeneChip assay was performed on RNA samples pooled from 4 to 5 lungs in each dietary group and therefore lack statistical data. The qRT-PCR data were obtained from a different cohort of mice treated identically to those used for obtaining the GeneChip data. $N = 4–5$ mice/group, $^{*}p = 0.02–0.05$, $^{**}p = 0.01–0.001$, $^{***}p < 0.0002$.

The template specific products generated by the PCR cycles are detected and quantified with an interchelating fluorescent dye.

Quantitative RT-PCR data show that 8 out of the 13 genes were induced in a dose dependent manner by increasing dietary α-T concentrations. These inductions were observed in both male and female lungs. All of the induced genes share a common biological function in maintaining cell shape and movement, *in vivo.* The cytoskeleton genes induced by dietary α-T included members of the myosin and troponin families, tropomyosin, α-actin, keratin complexes I and II, repetin and loricrin. Keratin complexes 1–13 and 2–4 have previously been shown to be co-regulated genes that encode proteins of the intermediate filaments in mammalian epithelial cells [56]. Repetin, another α-T induced gene in the lungs has also been suggested to be a component of intermediate filaments in keratinocytes [57]. Coordinated induction of the genes that play an important role in the cytoskeleton-intermediate filament functions of a cell is reminiscent of the induction of prokaryotic operons. The *Lac* operon was discovered as a cluster of three co-regulated *E. coli* genes activated by the supplementation of lactose to a bacterial growth medium deficient in lactose. In this study, we detected the induction of a cluster of 13 cytoskeleton genes in the lungs of mice in response to feeding diets supplemented with increasing concentrations of α-T. The coordinated induction of the affected, intron-containing, non-contiguous genes precludes them from being an authentic operon. However, 3 of the 13 genes were localized on chromosome 7 and another 3 on chromosome 11 suggesting that some of these α-T sensitive genes may be co-segregated.

Co-regulated genes often share common transcription factors. It has previously been shown that SRF, a transcription factor that has multiple co-regulators, is essential for the expression of smooth and cardiac muscle specific genes [58]. SRF binds to oligonucleotide motifs with $CC(A/T)_6GG$ sequence (CArG-box). We speculate that α-T may induce some of the cytoskeleton genes through the actions of SRF.

Cellular localization of proteins encoded by selected α-T sensitive genes

Figure 4 shows representative data from immunohistological analysis of lung tissues from three different female mice fed the assigned diets. The anti-tropomyosin antibody stained sections show the most notable increases in the lungs from mice fed the α-T-high diet. The staining appears to be primarily localized to the cytoplasmic regions of cells that would be occupied by type I pneumocytes.

Figure 4 also shows representative areas of bronchiolar regions from the lung sections from three different mice per group. Anti-myosin antibody stain appeared to be diffuse and localized to the epithelial layer. The staining intensity was highest in the bronchiole regions of lungs from the mice fed the α-T-high diet compared to those fed the α-T-poor diet. In contrast to the myosin stained regions of the bronchioles, anti-actin antibody stained regions that appeared to be less diffuse and primarily localized to sub-epithelial layers. The staining appeared to be least intense in the lungs of mice fed the α-T-poor diet and highest in the sections from mice fed the α-T-high diet.

Immunohistochemical analyses of the three encoded proteins suggest that these genes are expressed in lung

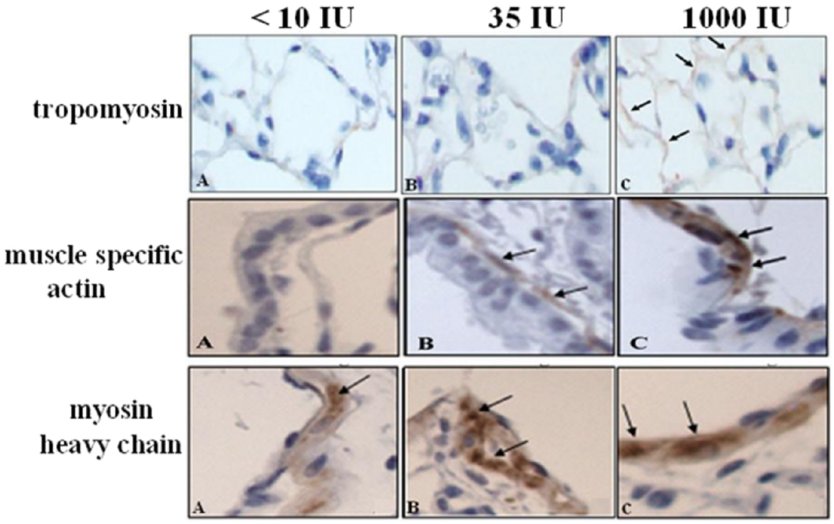


Figure 4. Induction of cytoskeleton proteins in the lungs of female mice fed increasing concentrations of α-T in the diet. All sections were lightly stained with hematoxylin to visualize the nuclei. The final magnification is X-400. Representative sections from three different lungs are shown. Top panel shows alveolar regions. Light brown deposits identify tropomyosin expression. The middle and the bottom panels show bronchiolar regions stained with anti-α-actin-Ab or with anti-smooth muscle myosin Ab, respectively. The dark brown regions identify areas of bronchioles where the antibodies were localized.

bronchioles and alveolar-capillary septae. The precise identity of the cells in the bronchioles, microvasculature and alveolar septae of the lungs that may express the α-T-sensitive cytoskeleton gene cluster remain to be more fully characterized. Previous studies have shown that the expression of these genes is restricted to smooth muscle cells [59–61]. In addition, members of the actin and myosin families are also expressed in endothelial cells [62] which make up to 50% of the cells in lung parenchyma. A more rigorous immunohistochemical analysis is necessary to discriminate between endothelial cells and pulmonary epithelial cells including alveolar wall pneumocytes. A recent study identified the induction of smooth muscle actin using anti-α-smooth muscle actin antibody in lung sections from mice subjected to repeated allergen exposure [63]. The immunohistological staining with muscle specific actin antibody in Figure 4 is similar to that seen in panels i–l of Figure 5 in a recent publication [63]. These comparisons suggest that chronic α-T-high in lungs may result in lung cytoarchitecture that has some features similar to that obtained by chronic exposures to allergen in mouse.

We have used commercially available antibodies for these studies and appreciate the possible limitations of the apparent correlations between the mRNA data and immunohistochemical data. Most of these antibodies are directed against the C-terminal epitopes of the protein and may react with multiple members of the same family of proteins. Therefore, more rigorous analysis with antibodies that are directed against the regions encoded by mRNA regions identified by the GeneChip or the qRT-PCR assays are necessary for defining the causal relationships between dietary α-T and changes in mRNA and protein expression and their cellular localization.

Summary

In summary, the expression of ~15,000 lung genes from the male and the female mice fed three different α-T containing diets were studied. Increase in dietary α-T that ranged two-orders of magnitude resulted in lung α-T concentrations that ranged 40-fold. More than 80 genes appeared to be sensitive to the α-T status of the lung tissue. Of these, 13 genes became the focus of this study because all of them were coordinately induced by increase in lung α-T concentrations in both male and female mice. The induction of most of the the co-regulated cytoskeleton genes detected by the GeneChip assay was independently confirmed by qRT-PCR and three of the encoded proteins were localized to regions of bronchioles and alveolar septae. The data from the genome-wide search suggest the possibility that α-T (or its metabolites including oxidative products) might influence various aspects of cytoskeleton biology. Future studies are needed to illustrate how the observation in lungs may affect lung functions and how the data may relate to such conditions as α-T deficiency related myopathies and neurodegenerative disorders.

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Appendix I. Genes sensitive to 1000 IU diet in female lungs.

			Signal		
Fold Δ	Gene title	Gene symbol	< 10 IU	1000 IU	Probe set ID
−59.7	Eukaryotic translation initiation factor 4, gamma 1	Eif4g1	44.7	0.7	1427037_at
−36.8	PHD finger protein 3	Phf3	44.8	1.3	1438070_at
−32.0	Serine (or cysteine) proteinase inhibitor, clade A, member 1a	Serpina1a	47.1	1	1420553_x_at
−29.9	Proteoglycan 2, bone marrow	Prg2	18.4	0.6	1422873_at
−26.0	Nuclear factor I/A	Nfia	19.2	0.8	1427733_a_at
−24.3	RAB2, member RAS oncogene family	Rab2	38.7	1.3	1418623_at
−24.3	Serine (or cysteine) proteinase inhibitor, clade A	Serpina3k	74.7	2.9	1423866_at
−22.6	Diabetic embryopathy 1	Dep1	17.4	0.4	1427602_at
−22.6	Formin binding protein 3	Fnbp3	37.4	1.6	1420917_at
−18.4	AF4/FMR2 family, member 1	Aff1	37.1	3	1425640_at
−18.4	Chemokine (C−X−C motif) ligand 9	Cxcl9	18	0.7	1418652_at
−17.1	Golgi phosphoprotein 3	Golph3	49.2	3.3	1420117_at
−17.1	Growth hormone	Gh	23.8	2.1	1460310_a_at
−16.0	Cornichon homolog 2 (Drosophila)	Cnih2	27.2	1	1417524_at
−16.0	Group specific component	Gc	71.8	5.7	1426547_at
−14.9	Peroxisomal acyl-CoA thioesterase 2B	MGI:2159621	17.9	1.1	1422077_at
−13.9	Ribosomal protein S6 kinase, polypeptide 2	Rps6ka2	25.6	4.6	1417543_at
−13.0	Actin, beta, cytoplasmic	Actb	1247	107.3	1419734_at
−13.0	Sulfotransferase family 2A, dehydroepiandrosterone	Sult2a2	23.5	1	1419528_at
−12.1	A disintegrin and metalloprotease domain 34	Adam34	11.5	0.6	1428030_at
−12.1	Germ cell-specific gene 2	Gsg2	13.4	0.6	1450886_at
−9.8	Developmental pluripotency-associated 3	Dppa3	21.5	2.7	1424295_at
−9.8	Protein tyrosine phosphatase, receptor type, K	Ptprk	46.5	3.7	1431680_a_at
−9.2	Similar to Cyp2c40 protein	LOC433247	39.5	3.2	1423244_at
−8.6	Arylacetamide deacetylase (esterase)	Aadac	19.9	2.3	1448813_at
−8.6	Hus1 homolog (S. pombe)	Hus1	41.3	4.1	1425366_a_at
−8.6	Proteasome (prosome, macropain) 26S subunit, non-ATP	Psmd11	28.7	1.6	1456104_at
−8.0	4-Hydroxyphenylpyruvic acid dioxygenase	Hpd	47.7	6.9	1424618_at
−8.0	Albumin 1	Alb1	699.9	83.7	1425260_at
−8.0	BTB (POZ) domain containing 4	Btbd4	25.1	1.1	1453856_at
−8.0	Solute carrier organic anion transporter family, member 1b2	Slco1b2	16.8	0.8	1452494_s_at
−7.5	Apolipoprotein A-II	Apoa2	244.9	33.8	1417950_a_at
−7.5	Phenylalanine hydroxylase	Pah	42.8	4.1	1454638_a_at
−7.5	Tripartite motif protein 8	Trim8	60.2	8.2	1438679_at
−7.0	Aldehyde dehydrogenase 2, mitochondrial	Aldh2	110.7	13.8	1434987_at
−7.0	Alpha-2-HS-glycoprotein	Ahsg	31.8	1.1	1455093_a_at
−7.0	Importin 8	Ipo8	29.5	4.2	1452185_at
−7.0	RFamide-related peptide	Rfrp	12.6	1.6	1421686_at
−7.0	Serine (or cysteine) proteinase inhibitor, clade A, member 1b	Serpina1b///Serpina1d///Serpina1e	137.3	17.3	1418282_x_at
−7.0	Topoisomerase (DNA) II beta	Top2b	40.9	4.9	1448458_at
−6.5	Cytochrome P450, family 3, subfamily a, polypeptide 41	Cyp3a41	35.9	6	1419704_at
−6.5	Ubiquitin C	Ubc	153.9	23	1454373_x_at
−6.1	Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	60.2	7.6	1421679_a_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
− 6.1	Fibroblast growth factor 9	Fgf9	34.8	2.9	1420795_at
− 5.7	Apolipoprotein C-III	Apoc3	58.9	10.3	1418278_at
− 5.7	B-cell leukemia/lymphoma 6	Bcl6	54.7	9.1	1450381_a_at
− 5.7	Breast carcinoma amplified sequence 3	Bcas3	18.3	3	1423528_at
− 5.7	G protein-coupled receptor 37	Gpr37	33.8	5.1	1450875_at
− 5.7	Mitogen activated protein kinase kinase kinase 3	Map3k3	35.5	6	1426687_at
− 5.7	Ribosomal protein L7	Rpl7	51.6	5.9	1427452_at
− 5.7	Serine (or cysteine) proteinase inhibitor, clade A, member 1a	Serpina1a	190.1	27.3	1448680_at
− 5.3	ATPase, class VI, type 11A	Atp11a	47.8	11.4	1421167_at
− 5.3	C-type lectin domain family 2, member h	Clec2h	25.3	3	1451438_s_at
− 5.3	Heat shock protein 1	Hspb1	138.1	22.6	1427853_a_at
− 5.3	Nephrosis 1 homolog, nephrin (human)	Nphs1	30.3	5.7	1422142_at
− 5.3	Rap guanine nucleotide exchange factor (GEF) 1	Rapgef1	72.7	11.7	1421146_at
− 5.3	Tumor necrosis factor receptor superfamily, member 19	Tnfrsf19	46.5	8.4	1415921_a_at
− 4.9	Apolipoprotein A-I	Apoa1	56.5	14.1	1419232_a_at
− 4.9	Brix domain containing 1	Bxdc1	75.8	14.1	1425884_at
− 4.9	Major urinary protein 5	Mup5	27.5	3.8	1426166_at
− 4.9	Myosin, heavy polypeptide 9, non-muscle	Myh9	2934.1	533.5	1420172_at
− 4.9	Neurotensin receptor 2	Ntsr2	16.6	0.9	1417151_a_at
− 4.9	Tubulin, beta 2	Tubb2	21.8	5.1	1427838_at
− 4.6	ATP-binding cassette, sub-family A (ABC1), member 1	Abca1	56.5	9	1450392_at
− 4.6	Interleukin 4 induced 1	Il4i1	29.8	8.4	1419192_at
− 4.6	O-Acetyltransferase	MGI:2384865	50.5	10.2	1427463_at
− 4.6	RAB27A, member RAS oncogene family	Rab27a	38.6	8.3	1425285_a_at
− 4.6	Sensitivity to red light reduced homolog (Arabidopsis)	Srr1	77.2	23.2	1456355_s_at
− 4.3	5 Hydroxytryptamine (serotonin) receptor 4	Htr4	20.1	5.3	1427654_a_at
− 4.3	A kinase (PRKA) anchor protein (yotiao) 9	Akap9	26	3.5	1455151_at
− 4.3	E74-like factor 2	Elf2	40.3	9.8	1428045_a_at
− 4.3	Protein kinase C, nu	Prkcn	35.1	8.5	1420567_at
− 4.3	Protein phosphatase 3, regulatory subunit B, alpha isoform (calcineurin B, type I)///expressed sequence AI553587	Ppp3r1///AI553587	23.9	4.4	1450368_a_at
− 4.3	Serine (or cysteine) proteinase inhibitor, clade C (antithrombin), member 1	Serpinc1	57.7	11.6	1417909_at
− 4.3	Sfi1 homolog, spindle assembly associated (yeast)	Sfi1	58.7	7.3	1452195_s_at
− 4.3	Syndecan 4	Sdc4	1586.2	326.9	1417654_at
− 4.3	Zinc finger protein 36, C3H type-like 2	Zfp36l2	40.9	9.6	1451034_at
− 4.0	Fructose bisphosphatase 1	Fbp1	60.7	11.5	1448470_at
− 4.0	G patch domain containing 2	Gpatc2	22.7	1.8	1420528_at
− 4.0	Plasminogen	Plg	32.5	8.3	1416729_at
− 4.0	Protein kinase, cAMP dependent, catalytic, beta	Prkacb	43.8	10.3	1420610_at
− 4.0	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 2	Stam2	30.9	5.7	1416975_at
− 4.0	Synaptogyrin 3	Syngr3	31.6	3.3	1416098_at
− 3.7	Adrenergic receptor, beta 3	Adrb3	36.1	8	1421555_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			<10 IU	1000 IU	
−3.7	Aldolase 2, B isoform	Aldob	71.4	10.5	1451194_at
−3.7	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit, isoform 1	Atp5a1	72.3	11.7	1420037_at
−3.7	G protein-coupled receptor kinase 6	Gprk6	34.3	9.4	1450480_a_at
−3.7	Galactosylceramidase	Galc	27.3	11.8	1420547_at
−3.7	High density lipoprotein (HDL) binding protein	Hdlbp	84.4	18	1449615_s_at
−3.7	Hydroxyprostaglandin dehydrogenase 15 (NAD)	Hpgd	149.8	43.6	1419906_at
−3.7	Nuclear factor I/C	Nfic	37	10.5	1422565_s_at
−3.7	Paxillin	Pxn	60.5	28.9	1426085_a_at
−3.7	Pregnancy zone protein	Pzp	52.3	14.5	1417246_at
−3.7	Purinergic receptor P2X-like 1, orphan receptor	P2rxl1	26.1	3.6	1450327_at
−3.7	Transthyretin	Ttr	93.7	19.8	1451580_a_at
−3.5	Alpha 1 microglobulin/bikunin	Ambp	26.5	7.6	1416649_at
−3.5	ATPase, class I, type 8B, member 1	Atp8b1	38.2	8.2	1420075_at
−3.5	LIM domain containing preferred translocation partner in lipoma	Lpp	147.8	28.6	1437801_at
−3.5	Methylcrotonoyl-coenzyme A carboxylase 2 (beta)	Mccc2	37.9	8.7	1428021_at
−3.5	Microrchidia 3	Morc3	21.5	6.6	1420092_at
−3.5	Protein kinase, cAMP dependent regulatory, type II alpha	Prkar2a	26.9	9	1427414_at
−3.5	STEAP family member 3	Steap3	19.5	5.3	1430355_a_at
−3.5	Transcription factor 20	Tcf20	40.6	14.4	1421909_at
−3.2	Ankyrin repeat domain 6	Ankrd6	22.7	8.1	1421386_at
−3.2	Bcl2-like 1	Bcl2l1	253.5	82.8	1420887_a_at
−3.2	Caspase 9	Casp9	50.9	13.9	1426125_a_at
−3.2	Crystallin, lamda 1	Cryl1	19.9	5.3	1430681_at
−3.2	DMRT-like family B with proline-rich C-terminal, 1	Dmrtb1	27.5	5	1427252_at
−3.2	Eukaryotic translation initiation factor 4A1	Eif4a1	971.8	269.8	1430980_a_at
−3.2	FMS-like tyrosine kinase 1	Flt1	51.1	24.9	1454037_a_at
−3.2	Golgi SNAP receptor complex member 1	Gosr1	25.6	6.1	1448256_at
−3.2	Growth factor receptor bound protein 10	Grb10	41.1	13.2	1425457_a_at
−3.2	Homeodomain interacting protein kinase 3	Hipk3	81.4	21.8	1419191_at
−3.2	Interferon activated gene 203///similar to interferon-inducible protein 203	Ifi203///LOC547362	22	6.4	1425008_a_at
−3.2	Linker of T-cell receptor pathways	Lnk	56.7	11.5	1450183_a_at
−3.2	Major urinary protein 1///major urinary protein 2	Mup1///Mup2	2173.2	671.1	1420465_s_at
−3.2	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	Nfkbia	140.6	36.3	1420089_at
−3.2	Phosphatidylinositol 3-kinase, C2 domain containing, alpha polypeptide	Pik3c2a	41.4	10.5	1425862_a_at
−3.2	Polycystic kidney disease 1 like 3	Pkd1l3	12.5	3.4	1444160_at
−3.2	Protein phosphatase 2, regulatory subunit B (B56), gamma isoform	Ppp2r5c	76.5	15.2	1425726_x_at
−3.2	Splicing factor, arginine/serine-rich 2 (SC-35)	Sfrs2	412.4	135.6	1452439_s_at
−3.2	Tetratricopeptide repeat domain 14	Ttc14	27.4	8.8	1432130_a_at
−3.2	v-crk sarcoma virus CT10 oncogene homolog (avian)-like	Crkl	46.2	12.6	1421954_at
−3.2	Zinc finger protein, autosomal///zinc finger protein X-linked	Zfa///Zfx	20.8	7.7	1422249_s_at
−3.0	Aldo-keto reductase family 1, member C6	Akr1c6	30.6	5.4	1417085_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			<10 IU	1000 IU	
−3.0	CASP8 and FADD-like apoptosis regulator	Cflar	96.2	27.6	1425686_at
−3.0	CUE domain containing 1	Cuedc1	26.4	7.4	1451447_at
−3.0	Endothelin 3	Edn3	17.7	5.6	1421136_at
−3.0	Eph receptor A3	Epha3	19.2	5	1425574_at
−3.0	Flavin containing monooxygenase 2	Fmo2	948.1	369.8	1422904_at
−3.0	Glioblastoma amplified sequence	Gbas	458.2	151.4	1419484_a_at
−3.0	Glucosaminyl (*N*-acetyl) transferase 2, I-branching enzyme	Gcnt2	34.3	11.6	1430826_s_at
−3.0	Glycerol-3-phosphate dehydrogenase 1 (soluble)	Gpd1	50.9	17.9	1416204_at
−3.0	Histocompatibility 13	H13	21.8	4.4	1428856_at
−3.0	Inositol hexaphosphate kinase 1	Ihpk1	125.9	45.8	1422968_at
−3.0	Nuclear factor I/C	Nfic	20.4	5.8	1427665_a_at
−3.0	Nuclear receptor subfamily 6, group A, member 1	Nr6a1	27.5	10.7	1421516_at
−3.0	Poliovirus receptor	Pvr	29.3	5.6	1450295_s_at
−3.0	Polymerase I and transcript release factor	Ptrf	73.6	29.8	1421431_at
−3.0	POU domain, class 2, transcription factor 1	Pou2f1	33.9	9	1427695_a_at
−3.0	Protein tyrosine phosphatase 4a2	Ptp4a2	551	148.8	1435129_at
−3.0	Rad and gem related GTP binding protein 1	Rem1	35.4	10.1	1450158_at
−3.0	Ras association (RalGDS/AF-6) domain family 3	Rassf3	75	27.9	1417015_at
−3.0	Secreted frizzled-related sequence protein 1	Sfrp1	22.7	9.8	1448395_at
−3.0	Soc-2 (suppressor of clear) homolog (C. elegans)	Shoc2	70.3	27.3	1425845_a_at
−3.0	Sphingosine phosphate lyase 1	Sgpl1	69.8	18.9	1415893_at
−3.0	SRY-box containing gene 4	Sox4	98.8	26.2	1419157_at
−3.0	Stomatin	Stom	107.1	42	1419098_at
−3.0	Thymosin, beta 10	Tmsb10	23.6	8.4	1436682_at
−3.0	Thyrotroph embryonic factor	Tef	102.1	34.9	1450184_s_at
−3.0	Transforming growth factor, beta receptor II	Tgfbr2	73.4	25.8	1425444_a_at
−3.0	Transmembrane protein 33	Tmem33	21.3	9.2	1428888_at
−3.0	Ubiquitin specific protease 12	Usp12	33.3	11.2	1427478_at
−3.0	utrophin	Utrn	102.5	41.7	1427569_a_at
−3.0	Wiskott–Aldrich syndrome-like (human)	Wasl	46.5	10.9	1426777_a_at
−2.8	cathepsin E	Ctse	112.3	36.7	1427797_s_at
−2.8	Cdc42 GTPase-activating protein	Cdgap	36.4	12.6	1450255_at
−2.8	Cullin 4A	Cul4a	72.3	26.3	1426060_at
−2.8	Cytochrome P450, family 1, subfamily a, polypeptide 1	Cyp1a1	30.9	11.1	1422217_a_at
−2.8	Ephrin B2	Efnb2	52.8	18.6	1449549_at
−2.8	Epithelial stromal interaction 1 (breast)	Epsti1	28.7	13.5	1432235_at
−2.8	Fragile X mental retardation syndrome 1 homolog	Fmr1	62.6	11.2	1426086_a_at
−2.8	Glycoprotein galactosyltransferase alpha 1, 3	Ggta1	82.6	21.6	1451843_a_at
−2.8	Hypoxia up-regulated 1	Hyou1	229.2	106.5	1423291_s_at
−2.8	Ig rearranged heavy chain (NC12-H7) mRNA VH-DH-JH1 region	–	24	8.1	1452535_at
−2.8	Immunoglobulin heavy chain 4 (serum IgG1)	Igh-4	20.4	5.2	1427756_x_at
−2.8	Linker for activation of T cells family, member 2	Lat2	41.6	13.4	1426169_a_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
− 2.8	Max protein	Max	73.2	19.3	1423501_at
− 2.8	Nuclear receptor subfamily 3, group C, member 1	Nr3c1	73.9	26.2	1421866_at
− 2.8	Pentatricopeptide repeat domain 1	Ptcd1	58.9	20.4	1416391_at
− 2.8	Pinin	Pnn	66.4	23.4	1423325_at
− 2.8	Potassium inwardly-rectifying channel, subfamily J, member 3	Kcnj3	35.7	10.4	1421468_at
− 2.8	Serine hydrolase-like	Serhl	63.3	25.5	1434145_s_at
− 2.8	Stomatin	Stom	13	4	1452522_at
− 2.8	Suppressor of Ty 16 homolog (S. cerevisiae)	Supt16h	848.2	237.5	1419741_at
− 2.8	Tissue inhibitor of metalloproteinase 2	Timp2	308.9	96.6	1420924_at
− 2.8	Tissue inhibitor of metalloproteinase 3	Timp3	269.4	109.6	1419088_at
− 2.8	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase	B4galt1	1056.4	270	1418014_a_at
− 2.6	3-Monooxygenase/tryptophan 5-monooxygenase activation	Ywhag	43.5	17	1420816_at
− 2.6	CUG triplet repeat, RNA binding protein 1	Cugbp1	41.1	14.2	1425932_a_at
− 2.6	DnaJ (Hsp40) homolog, subfamily B, member 5	Dnajb5	32.3	11	1421961_a_at
− 2.6	Dynamin 2	Dnm2	85.1	34	1432004_a_at
− 2.6	Fibroblast growth factor receptor 3	Fgfr3	99.6	33.8	1425796_a_at
− 2.6	Glial cell line derived neurotrophic factor family receptor alpha	Gfra4	22.6	9.6	1450835_a_at
− 2.6	Glycerol-3-phosphate acyltransferase, mitochondrial	Gpam	95	28.4	1425834_a_at
− 2.6	Histocompatibility 2, T region locus 24	H2-T24	44.4	19.1	1422160_at
− 2.6	Implantation serine protease 2	Isp2	29.8	16.6	1449805_at
− 2.6	Jerky	Jrk	28.7	6.4	1418410_at
− 2.6	Lecithin-retinol acyltransferase	Lrat	89.7	30.6	1421345_at
− 2.6	LIM and SH3 protein 1	Lasp1	98.2	27.2	1448207_at
− 2.6	Motile sperm domain containing 2	Mospd2	96	71.9	1453920_a_at
− 2.6	Multiple substrate lipid kinase	Mulk	47.9	18.3	1431655_a_at
− 2.6	Murine retrovirus readthrough RNA sequence	–	24.7	11.5	1427827_at
− 2.6	Podocalyxin-like	Podxl	104.5	37.2	1417396_at
− 2.6	Protein kinase, interferon-inducible double stranded RNA dependent	Prkr	52.6	17.1	1422006_at
− 2.6	Retinoschisis 1 homolog (human)	Rs1h	25.8	13.9	1421085_at
− 2.6	Sec61 alpha 1 subunit (S. cerevisiae)	Sec61a1	176.9	69.3	1416190_a_at
− 2.6	Sel1 (suppressor of lin-12) 1 homolog (C. elegans)	Sel1h	64.9	18.4	1453559_a_at
− 2.6	Spleen tyrosine kinase	Syk	31.2	10.3	1425797_a_at
− 2.6	Stearoyl-coenzyme A desaturase 2	Scd2	188.3	95.4	1415824_at
− 2.6	Stomatin	Stom	114.8	34.3	1419099_x_at
− 2.6	Synaptotagmin XI	Syt11	32.7	13.3	1449264_at
− 2.6	Thyroid hormone receptor associated protein 3	Thrap3	91.1	29.3	1427408_a_at
− 2.6	Thyroid transcription factor 1	Titf1	101.4	28.2	1422346_at
− 2.6	Twisted gastrulation homolog 1 (Drosophila)	Twsg1	172.8	58	1426179_a_at
− 2.6	Zinc finger protein 146	Zfp146	40.2	17.2	1422135_at
− 2.6	Zinc finger protein 64	Zfp64	37.9	17.1	1430117_a_at
− 2.5	ADP-ribosylation factor 2	Arf2	43.4	12.3	1456473_x_at
− 2.5	Apolipoprotein B	Apob	25.6	6.9	1455593_at

Appendix I – *Continued*

			Signal		
Fold Δ	Gene title	Gene symbol	< 10 IU	1000 IU	Probe set ID
− 2.5	Beta galactoside alpha 2,6 sialyltransferase 1	St6gal1	71.9	28.1	1420927_at
− 2.5	Bone morphogenetic protein 1	Bmp1	40.9	18.7	1427457_a_at
− 2.5	Claudin 18	Cldn18	602.9	222.2	1425445_a_at
− 2.5	Coronin, actin binding protein 1C	Coro1c	38.5	17.7	1419911_at
− 2.5	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2	Ctdsp2	103.1	42.4	1423660_at
− 2.5	Cytochrome b-245, beta polypeptide	Cybb	98.3	28.7	1422978_at
− 2.5	Dynamin 1-like	Dnm1l	35.1	13	1452638_s_at
− 2.5	Ets variant gene 5	Etv5	102.6	33.1	1420998_at
− 2.5	F-box and WD-40 domain protein 11	Fbxw11	46.3	19.3	1425461_at
− 2.5	FERM domain containing 4B	Frmd4b	72.2	28.9	1426331_a_at
− 2.5	Fibrinogen, alpha polypeptide	Fga	39.5	17	1424279_at
− 2.5	Gap junction membrane channel protein alpha 1	Gja1	102.5	43.3	1415801_at
− 2.5	Guanine nucleotide binding protein, alpha 12	Gna12	67.1	28.4	1421026_at
− 2.5	Insulin degrading enzyme	Ide	82.2	34.4	1453988_a_at
− 2.5	Kruppel-like factor 9	Klf9	52	22.3	1436952_at
− 2.5	Membrane associated guanylate kinase	Magi3	81.5	27.6	1421035_a_at
− 2.5	Membrane-spanning 4-domains, subfamily A, member 6C	Ms4a6c	59.2	25	1450234_at
− 2.5	MOB1, Mps One Binder kinase activator-like 1B (yeast)	Mobk1b	213.3	84.2	1424484_at
− 2.5	Myosin 1H	Myo1h	46.9	15.7	1450543_at
− 2.5	Myotubularin related protein 1	Mtmr1	60.5	24.4	1421879_at
− 2.5	Orosomucoid 1	Orm1	24.9	10.4	1451054_at
− 2.5	Pituitary tumor-transforming 1 interacting protein	Pttg1ip	26.1	11.6	1420132_s_at
− 2.5	Polycomb group ring finger 2	Pcgf2	40.9	18.1	1420645_at
− 2.5	Polymerase (RNA) III (DNA directed) polypeptide C	Polr3c	45.5	34.1	1451658_a_at
− 2.5	RAB5B, member RAS oncogene family	Rab5b	69.6	22.9	1422119_at
− 2.5	Sec61 alpha 1 subunit (S. cerevisiae)	Sec61a1	209.3	91.7	1416191_at
− 2.5	ST8 alpha-*N*-acetyl-neuraminide alpha-2,8-sialyltransferase 4	St8sia4	34.8	13.6	1430391_a_at
− 2.5	Superoxide dismutase 1, soluble	Sod1	3545.5	1418	1451124_at
− 2.5	T-box 15	Tbx15	13.1	7.2	1422195_s_at
− 2.5	Toll interacting protein	Tollip	99	29.5	1423048_a_at
− 2.5	*Trans*-golgi network protein	Tgoln1	163.9	69.5	1423308_at
− 2.5	Ubiquitin specific protease 22	Usp22	58.7	21.6	1456043_at
− 2.3	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	Pfkfb3	64.7	18.1	1456676_a_at
− 2.3	A kinase (PRKA) anchor protein 2	Akap2	230.1	104	1449168_a_at
− 2.3	Adaptor-related protein complex 3, mu 1 subunit	Ap3m1	122.1	47.7	1448309_at
− 2.3	Adenylate kinase 3	Ak3	99.1	37	1432436_a_at
− 2.3	ADP-ribosylation factor 3	Arf3	45.3	16.3	1421789_s_at
− 2.3	Angiopoietin 1	Angpt1	103.2	49	1421441_at
− 2.3	Ceruloplasmin	Cp	220.8	95.4	1417496_at
− 2.3	Chemokine binding protein 2	Ccbp2	57.5	21.9	1422111_at
− 2.3	Cullin 4A	Cul4a	52.8	22.6	1426061_x_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
− 2.3	Endothelial PAS domain protein 1	Epas1	461	187.9	1449888_at
− 2.3	Erythrocyte protein band 4.1-like 2	Epb4.1l2	119.2	37.4	1433492_at
− 2.3	Glial cell line derived neurotrophic factor family receptor alpha 1	Gfra1	19.1	6.9	1450440_at
− 2.3	Glycoprotein 38	Gp38	1881.6	920	1419309_at
− 2.3	Histone1, H3d	Hist1h3d	18.2	8.1	1427863_at
− 2.3	Iduronate 2-sulfatase	Ids	41	9.9	1421216_a_at
− 2.3	Inositol 1,4,5-triphosphate receptor 2	Itpr2	21.9	9.7	1427693_at
− 2.3	Interleukin 6 signal transducer	Il6st	133.5	46.8	1421239_at
− 2.3	Janus kinase 1	Jak1	30.5	14.9	1433804_at
− 2.3	Kit ligand	Kitl	60	29.9	1426152_a_at
− 2.3	Lin 7 homolog c (C. elegans)	Lin7c	97.6	54.9	1449262_s_at
− 2.3	MAD homolog 5 (Drosophila)	Smad5	68.8	25.6	1421047_at
− 2.3	Myc target 1	Myct1	45.6	20.6	1426433_at
− 2.3	Neural precursor cell expressed, developmentally down-regulted gene 4	Nedd4	317.6	123.8	1421955_a_at
− 2.3	Notch gene homolog 2 (Drosophila)	Notch2	1000.6	333.9	1455556_at
− 2.3	Nuclear receptor co-repressor 1	Ncor1	202	72.3	1423201_at
− 2.3	Peptidyl-prolyl isomerase G (cyclophilin G)	Ppig	62.3	23	1436505_at
− 2.3	Protein kinase, AMP-activated, beta 1 non-catalytic subunit	Prkab1	103.4	30.8	1452457_a_at
− 2.3	Ring finger protein 14	Rnf14	77.4	30	1431030_a_at
− 2.3	SEC14-like 1 (S. cerevisiae)	Sec14l1	114	48.2	1451908_a_at
− 2.3	UDP-*N*-acetylglucosamine (UDP-GlcNAc) transporter	Slc35a3	39	16.2	1424580_at
− 2.3	Spindle pole body component 25 homolog (S. cerevisiae)	Spbc25	18.6	8.3	1453815_at
− 2.3	Stomatin	Stom	164.8	45.5	1449341_a_at
− 2.3	SWI/SNF related, matrix associated	Smarcd2	36.2	16.4	1426192_at
− 2.3	Thrombospondin, type I domain containing 6	Thsd6	38.5	16.6	1420336_at
− 2.3	Transformation related protein 53	Trp53	48.7	24	1426538_a_at
− 2.3	Transformation related protein 63	Trp63	20	2.6	1452027_a_at
− 2.3	Transforming growth factor, beta receptor I	Tgfbr1	19.1	8.3	1420894_at
− 2.3	Transforming growth factor, beta receptor III	Tgfbr3	72.8	30.3	1425620_at
− 2.3	Translocated promoter region	Tpr	110	54.5	1456112_at
− 2.3	Zinc finger protein 191	Zfp191	38.1	18.4	1422810_at
− 2.3	Zinc finger protein 207	Zfp207	476.6	247.9	1438714_at
− 2.3	Zinc finger protein 346	Zfp346	48.9	20.4	1448581_at
− 2.1	5′-Nucleotidase, cytosolic II	Nt5c2	28	13.3	1448614_at
− 2.1	Activating transcription factor 2	Atf2	71	32.9	1427559_a_at
− 2.1	Adaptor protein complex AP-2, alpha 2 subunit	Ap2a2	404.3	174.4	1435869_s_at
− 2.1	Amyloid beta (A4) precursor-like protein 2	Aplp2	255.2	107.8	1432344_a_at
− 2.1	Arginine-tRNA-protein transferase 1	Ate1	56.8	23.1	1420652_at
− 2.1	Argininosuccinate synthetase 1	Ass1	133.3	62.3	1416239_at
− 2.1	Aryl hydrocarbon receptor nuclear translocator	Arnt	41.5	22.3	1421721_a_at
− 2.1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	Atp6v0a1	43.1	21.3	1460650_at
− 2.1	ATP-binding cassette, sub-family C (CFTR/MRP), member 5	Abcc5	149.9	60.7	1435684_at

Appendix I – *Continued*

			Signal		
Fold Δ	Gene title	Gene symbol	<10 IU	1000 IU	Probe set ID
−2.1	B-cell leukemia/lymphoma 11B	Bcl11b	33.8	12.8	1450339_a_at
−2.1	Cadherin 5	Cdh5	97.4	31.5	1422047_at
−2.1	Caldesmon 1	Cald1	165.1	61.2	1424770_at
−2.1	Carboxypeptidase D	Cpd	129.9	56.7	1418018_at
−2.1	CEA-related cell adhesion molecule 1	Ceacam1///Ceacam2	183	79	1422123_s_at
−2.1	Ceroid-lipofuscinosis, neuronal 8	Cln8	45.5	17.5	1448456_at
−2.1	Cingulin-like 1	Cgnl1	64.1	23.3	1421158_at
−2.1	Coxsackievirus and adenovirus receptor	Cxadr	57.2	36.7	1449949_a_at
−2.1	CXXC finger 1 (PHD domain)	Cxxc1	40.6	17	1454106_a_at
−2.1	Deleted in liver cancer 1	Dlc1	36.2	17.4	1450206_at
−2.1	DnaJ (Hsp40) homolog, subfamily C, member 5	Dnajc5	68.4	27.7	1448851_a_at
−2.1	Eukaryotic translation initiation factor 3, subunit 8	Eif3s8	209.8	108.8	1415859_at
−2.1	Eukaryotic translation initiation factor 4A1	Eif4a1	1101.2	719.5	1427058_at
−2.1	Frizzled homolog 1 (Drosophila)	Fzd1	46.8	22	1422985_at
−2.1	GPI-anchored membrane protein 1	Gpiap1	157.2	63.2	1448348_at
−2.1	Hemopexin	Hpxn	64	22.3	1423944_at
−2.1	Histone 1, H2ad	Hist1h2ad	253.6	148.9	1438009_at
−2.1	histone 2, H3c1	Hist2h3c1///Hist2h3c2///Hist1h3g///Hist1h3f///Hist1h3c///Hist1h3d///Hist1h3b///Hist1h3e///Hist1h3h///Hist1h3i///Hist2h3b///Hist1h3a	84.1	36.9	1460314_s_at
−2.1	Huntingtin interacting protein 1	Hip1	98.2	43	1424755_at
−2.1	Inner membrane protein, mitochondrial	Immt	78.7	66.4	1429533_at
−2.1	Insulin-like growth factor I receptor	Igf1r	73.2	42.8	1426565_at
−2.1	Interferon regulatory factor 3	Irf3	34.2	11.2	1438721_a_at
−2.1	Kelch-like ECH-associated protein 1	Keap1	64.3	27	1450747_at
−2.1	Large tumor supressor	Lats1	17.4	9.2	1427679_at
−2.1	LIM and senescent cell antigen-like domains 1	Lims1	194.4	80.8	1418231_at
−2.1	Mevalonate kinase	Mvk	49.5	27.7	1430619_a_at
−2.1	*N*-Deacetylase/*N*-sulfotransferase (heparan glucosaminyl) 1	Ndst1	59.2	20.6	1422044_at
−2.1	Nebulette	Nebl	56.1	29.3	1451846_at
−2.1	Nucleoplasmin 3	Npm3	63.6	24.1	1423522_at
−2.1	PHD finger protein 17	Phf17	62.9	26.8	1426753_at
−2.1	Pleckstrin homology, Sec7 and coiled-coil domains 3	Pscd3	170.4	65.7	1431707_a_at
−2.1	Procollagen–proline, 2-oxoglutarate 4-dioxygenase	P4ha1	108.6	53	1426519_at
−2.1	Prosaposin	Psap	965.8	348.9	1421813_a_at
−2.1	Protein phosphatase 2, regulatory subunit B	Ppp2r2b	34.3	17.1	1426621_a_at
−2.1	RAD23a homolog (S. cerevisiae)	Rad23a	120.5	51.4	1453623_a_at
−2.1	Retinol dehydrogenase 7	Rdh7	23.2	10.6	1448723_at
−2.1	Rho guanine nucleotide exchange factor (GEF) 12	Arhgef12	35.8	17	1453853_a_at
−2.1	Rho/rac guanine nucleotide exchange factor (GEF) 2	Arhgef2	90.9	36.6	1427646_a_at
−2.1	Sema domain, immunoglobulin domain (Ig)	Sema3a	20.3	8.5	1420417_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
−2.1	Sfi1 homolog, spindle assembly associated (yeast)	Sfi1	130	69.8	1426787_at
−2.1	Similar to immunoglobulin light chain variable region	LOC243469///LOC435905	89.4	44	1425738_at
−2.1	Solute carrier family 13 member 3	Slc13a3	26	19	1438377_x_at
−2.1	Solute carrier family 23 (nucleobase transporters), member 2	Slc23a2	45.7	18.8	1417330_at
−2.1	Solute carrier family 25, member 30	Slc25a30	68.5	30	1420835_at
−2.1	Splicing factor, arginine/serine-rich 2, interacting protein	Sfrs2ip	55.5	25.7	1452885_at
−2.1	Synaptotagmin binding, cytoplasmic RNA interacting protein	Syncrip	89	36.5	1422769_at
−2.1	Telomeric repeat binding factor 1	Terf1	19.4	8.9	1431332_a_at
−2.1	Tetratricopeptide repeat domain 1	Ttc1	71.6	38.7	1416994_at
−2.1	Transferrin receptor	Tfrc	62.3	34.1	1422966_a_at
−2.1	Tripartite motif protein 8	Trim8	88.3	61.1	1460237_at
−2.1	Vinculin	Vcl	134.9	68	1416157_at
−2.1	Yip1 domain family, member 2	Yipf2	120.3	48.3	1420668_a_at
−2.1	ZW10 interactor	Zwint	27.2	13.2	1427540_at
−2.0	Adaptor-related protein complex 3, beta 1 subunit	Ap3b1	89.3	54.3	1450915_at
−2.0	Adducin 3 (gamma)	Add3	233.7	144.1	1426574_a_at
−2.0	Ariadne homolog 2 (drosophila)	Arih2	38.7	19	1418523_at
−2.0	AT rich interactive domain 4B (Rbp1 like)	Arid4b	39.1	13	1460384_a_at
−2.0	AT rich interactive domain 5B (Mrf1 like)	Arid5b	41.3	20.9	1420973_at
−2.0	Bromodomain adjacent to zinc finger domain, 1B	Baz1b	79.2	42	1420975_at
−2.0	BTB (POZ) domain containing 3	Btbd3	73.4	42.2	1425660_at
−2.0	CCCTC-binding factor	Ctcf	87.5	41.2	1449042_at
−2.0	CDP-diacylglycerol synthase 2	Cds2	43	20.4	1418795_at
−2.0	Chloride channel 7	Clcn7	71.5	25	1450408_at
−2.0	Chromosome 9 open reading frame 102-like	Sr278	45.4	28.9	1452573_a_at
−2.0	Cortactin	Cttn	125.6	71.1	1421313_s_at
−2.0	DEAH (Asp-Glu-Ala-His) box polypeptide 36	Dhx36	79	45.1	1424398_at
−2.0	Diaphorase 1 (NADH)	Dia1	372.6	170.5	1425329_a_at
−2.0	Dynactin 4	Dctn4	468.1	217.5	1420861_at
−2.0	EH-domain containing 2	Ehd2	450.4	229.6	1424157_at
−2.0	Enhancer of zeste homolog 1 (Drosophila)	Ezh1	45.8	33.4	1418290_a_at
−2.0	Fetal Alzheimer antigen	Falz	291.6	138.7	1427310_at
−2.0	Ganglioside-induced differentiation-associated-protein 10	Gdap10	129.4	72.2	1420342_at
−2.0	Glia maturation factor, beta	Gmfb	123.3	80.8	1417069_a_at
−2.0	Growth arrest specific 7	Gas7	15.4	7.1	1417859_at
−2.0	Guanine nucleotide binding protein, alpha 13	Gna13	86.5	43.7	1453470_a_at
−2.0	Host cell factor C1	Hcfc1	52.3	28.8	1450439_at
−2.0	Hydroxysteroid (17-beta) dehydrogenase 12	Hsd17b12	319.3	139.1	1450010_at
−2.0	Immunoglobulin heavy chain (gamma polypeptide)	Ighg	38.5	26	1426174_s_at
−2.0	Immunoglobulin heavy chain (J558 family)	Igh-VJ558	28.6	11.6	1427852_x_at
−2.0	Immunoglobulin kappa chain, constant region	Igk-C	52	22.1	1452557_a_at
−2.0	Kinesin family member 1C	Kif1c	29.1	15.1	1426325_at

Appendix I – *Continued*

			Signal		
Fold Δ	Gene title	Gene symbol	< 10 IU	1000 IU	Probe set ID
−2.0	Kinesin family member 3B	Kif3b	60.5	41.3	1450074_at
−2.0	Kinesin-like 8	Knsl8	53.1	28.4	1425547_a_at
−2.0	Kruppel-like factor 3 (basic)	Klf3	90.8	41.3	1421604_a_at
−2.0	Membrane-bound transcription factor protease, site 1	Mbtps1	36.6	16.7	1431385_a_at
−2.0	Mitogen activated protein kinase 14	Mapk14	102.9	50.1	1416704_at
−2.0	Mitogen activated protein kinase 8 interacting protein 1	Mapk8ip1	37.7	20	1425679_a_at
−2.0	Moesin	Msn	834.1	350.4	1450379_at
−2.0	Nitrogen fixation gene 1 (S. cerevisiae)	Nfs1	63.1	28.3	1431431_a_at
−2.0	Non-POU-domain-containing, octamer binding protein	Nono	75.6	45	1431239_at
−2.0	Nuclear receptor subfamily 2, group C, member 2	Nr2c2	46.5	21.6	1451569_at
−2.0	O-linked *N*-acetylglucosamine (GlcNAc) transferase	Ogt	62.3	35.5	1436780_at
−2.0	Optic atrophy 1 homolog (human)	Opa1	52.1	21.3	1418768_at
−2.0	Polycomb group ring finger 6	Pcgf6	27.9	6.8	1454120_a_at
−2.0	Ral GEF with PH domain and SH3 binding motif 1	Ralgps1	72.4	32.7	1450085_at
−2.0	Ring finger protein 144	Rnf144	51.5	20.6	1421243_at
−2.0	S-adenosylhomocysteine hydrolase-like 1	Ahcyl1	119.3	56.7	1425576_at
−2.0	Stearoyl-coenzyme A desaturase 1	Scd1	394.6	171.6	1415965_at
−2.0	Superoxide dismutase 2, mitochondrial	Sod2	28.3	14.3	1417194_at
−2.0	Superoxide dismutase 3, extracellular	Sod3	358.1	158.6	1417634_at
−2.0	Syndecan 1	Sdc1	177.7	90.3	1448158_at
−2.0	*t*-Complex-associated-testis-expressed 1-like	Tcte1l	345.8	163	1449928_at
−2.0	Tripartite motif-containing 35	Trim35	76.4	34.3	1425621_at
−2.0	Tubulin, beta 5	Tubb5	782.9	464.8	1455719_at
−2.0	Vacuolar protein sorting 35	Vps35	76.5	40.3	1415784_at
−2.0	Vascular endothelial growth factor A	Vegfa	276.9	141.6	1451959_a_at
−2.0	Wee 1 homolog (S. pombe)	Wee1	46.8	17.8	1416774_at
−2.0	Zinc finger protein 125	Zfp125	38.5	13.9	1427536_at
2.0	3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	Hmgcs1	465.5	915.2	1433445_x_at
2.0	Anthrax toxin receptor 1	Antxr1	425	788.5	1451446_at
2.0	Apurinic/apyrimidinic endonuclease 1	Apex1	100.4	238.2	1437715_x_at
2.0	ATPase, H + transporting, lysosomal accessory protein 2	Atp6ap2	894.5	1605.7	1437688_x_at
2.0	CCR4-NOT transcription complex, subunit 4	Cnot4	87.6	161.3	1436645_a_at
2.0	CDP-diacylglycerol-inositol 3-phosphatidyltransferase (phosphatidylinositol synthase)	Cdipt	619.2	1156	1436715_s_at
2.0	CUG triplet repeat, RNA binding protein 2	Cugbp2	213.3	347.2	1451154_a_at
2.0	Desmoplakin	Dsp	209.1	407.6	1435494_s_at
2.0	Diabetic nephropathy-like protein (Dnr12) mRNA, partial sequence	–	242.6	511.3	1434380_at
2.0	F-box only protein 22	Fbxo22	91.5	177.7	1426593_a_at
2.0	FUS interacting protein (serine-arginine rich) 1	Fusip1	114.6	182.7	1423982_at
2.0	Histone deacetylase 2	Hdac2	99.2	203.5	1449080_at
2.0	MARCKS-like protein	Mlp	57.1	114.8	1437226_x_at
2.0	Mitogen-activated protein kinase kinase kinase kinase 4	Map4k4	67.9	143.6	1422615_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
2.0	Myoglobin	Mb	277.4	772.4	1451203_at
2.0	Neogenin	Neo1	105.8	208.8	1434931_at
2.0	Nuclear transcription factor, X-box binding 1	Nfx1	68.5	132.7	1428248_at
2.0	Nucleosome assembly protein 1-like 1	Nap1l1	190.2	367.1	1420477_at
2.0	Profilin 2	Pfn2	50.8	108.8	1436993_x_at
2.0	Protein tyrosine phosphatase, non-receptor type 21	Ptpn21	80.5	189.4	1419054_a_at
2.0	Proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)	Prg4	20.2	57.9	1449824_at
2.0	Protocadherin alpha 4///protocadherin alpha 6///protocadherin alpha 7///protocadherin alpha 5///protocadherin alpha 11///protocadherin alpha 10///protocadherin alpha 1///protocadherin alpha 9///protocadherin alpha 3///protocadherin alpha 12///protocadherin alpha 2///protocadherin alpha 8///protocadherin alpha subfamily C, 1///protocadherin alpha subfamily C, 2	Pcdha4///Pcdha6///Pcdha7///Pcdha5///Pcdha11///Pcdha10///Pcdha1///Pcdha9///Pcdha3///Pcdha12///Pcdha2///Pcdha8///Pcdhac1///Pcdhac2	45	65.7	1420798_s_at
2.0	RAS related protein 1b	Rap1b	328.9	598.7	1455349_at
2.0	Small nuclear ribonucleoprotein polypeptide A'	Snrpa1	99.2	219.1	1417351_a_at
2.0	Special AT-rich sequence binding protein 1	Satb1	39.5	75.5	1416008_at
2.0	Testis expressed gene 10	Tex10	141.8	250	1439464_s_at
2.0	Ubiquitin-like 1 (sentrin) activating enzyme E1B	Uble1b	357.7	632.3	1448283_a_at
2.0	UDP-glucose ceramide glucosyltransferase	Ugcg	58.5	160.4	1435133_at
2.0	Zinc finger protein 238	Zfp238	131.3	280.1	1417010_at
2.1	Aldehyde dehydrogenase family 3, subfamily A1	Aldh3a1	70.1	149.6	1418752_at
2.1	Dendritic cell protein GA17	Ga17	11.5	38	1437189_x_at
2.1	Ysophosphatidic acid G-protein-coupled receptor, 2	Edg2	95.2	152.9	1417143_at
2.1	Farnesyl diphosphate farnesyl transferase 1	Fdft1	164.9	322.4	1438322_x_at
2.1	High mobility group nucleosomal binding domain 3	Hmgn3	100.5	229.9	1434875_a_at
2.1	Malignant fibrous histiocytoma amplified sequence 1	Mfhas1	148.8	296.6	1429005_at
2.1	Nuclear receptor subfamily 2, group F, member 1	Nr2f1	17.6	40.9	1418157_at
2.1	Protein phosphatase 2a, catalytic subunit, alpha isoform	Ppp2ca	109.8	272.9	1456390_at
2.1	Reduced expression 3	Rex3	32.2	66.5	1448595_a_at
2.1	Secretory leukocyte protease inhibitor	Slpi	255	580.9	1448377_at
2.1	Serologically defined colon cancer antigen 33	Sdccag33	98.6	205.1	1427233_at
2.1	Sphingosine-1-phosphate phosphatase 1	Sgpp1	59.1	128.7	1420822_s_at
2.1	Suppressor of cytokine signaling 5	Socs5	106.2	230.2	1423350_at
2.1	Surfeit gene 4	Surf4	80.5	153.4	1455822_x_at
2.1	Transducer of ERBB2, 2	Tob2	159.9	278.3	1448666_s_at
2.1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase	B3galt3	45.2	88.5	1418736_at
2.1	Zinc finger proliferation 1	Zipro1	71.4	174.8	1449732_at
2.1	Zinc finger protein 654	Zfp654	35.7	68.2	1427969_s_at
2.3	Dishevelled, dsh homolog 1 (Drosophila)	Dvl1	277.9	548.2	1437301_a_at
2.3	Fibromodulin	Fmod	41.2	80.5	1456084_x_at
2.3	Glutamate receptor, ionotropic	Grina	157.2	305.3	1436297_a_at
2.3	Microtubule-associated protein, RP/EB family, member 2	Mapre2	435.7	816.5	1451989_a_at

Appendix I – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	1000 IU	
2.3	Minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	Mcm6	23.4	48.3	1438852_x_at
2.3	Nestin	Nes	131.1	200.7	1449022_at
2.3	Praja1, RING-H2 motif containing	Pja1	33.3	91.8	1452083_a_at
2.3	RAP2C, member of RAS oncogene family	Rap2c	98.1	212.4	1460430_at
2.3	Tripartite motif protein 27	Trim27	125	328.8	1456375_x_at
2.3	WD repeat domain 57 (U5 snRNP specific)	Wdr57	12.7	98.7	1452713_a_at
2.5	Deoxycytidine kinase	Dck	49.2	80	1439012_a_at
2.5	Nuclear casein kinase and cyclin-dependent kinase substrate 1	Nucks1	59	157.2	1444952_a_at
2.5	Schlafen 4	Slfn4	30.2	64.3	1427102_at
2.5	Sestrin 1	Sesn1	41.2	112.3	1438931_s_at
2.5	Small proline-rich protein 1A	Sprr1a	10.7	30.1	1449133_at
2.5	Tetraspanin 8	Tspan8	71.5	140.6	1420017_at
2.5	Tropomyosin 2, beta	Tpm2	15.3	64.3	1449577_x_at
2.6	Isopentenyl-diphosphate delta isomerase	Idi1	28.5	73.8	1423804_a_at
2.6	Kelch-like 2, Mayven (Drosophila)	Klhl2	60.4	148.9	1426978_at
2.6	Necdin	Ndn	59.6	171.6	1435382_at
2.8	Creatine kinase, muscle	Ckm	55.2	203.7	1417614_at
2.8	Tetratricopeptide repeat domain 19	Ttc19	8.3	30.5	1427114_at
3.2	PDZ and LIM domain 5	Pdlim5	18.2	64	1429783_at
3.7	Myosin, heavy polypeptide 1, skeletal muscle, adult	Myh1	13.2	71.4	1427520_a_at
4.3	Defensin beta 4	Defb4	7.6	31.9	1419600_at
4.9	Gamma-aminobutyric acid (GABA-B) receptor, 1	Gabbr1	9.8	67.4	1437188_at
4.9	Myosin, heavy polypeptide 8, skeletal muscle, perinatal	Myh8	9.5	63.6	1426650_at
5.7	Troponin I, skeletal, fast 2	Tnni2	42.3	190.9	1416889_at
6.5	Myosin light chain, phosphorylatable, fast skeletal muscle	Mylpf	25.5	146.1	1448371_at
6.5	Serine (or cysteine) proteinase inhibitor, clade B	Serpinb11	4.4	27.2	1449451_at
7.0	Adenosine deaminase	Ada	45.9	159.9	1417976_at
7.0	H19 fetal liver mRNA	H19	14	89.7	1448194_a_at
8.0	Actin, alpha 1, skeletal muscle	Acta1	76.4	434.4	1427735_a_at
8.6	Myosin, light polypeptide 3	Myl3	14.8	154.6	1427768_s_at
8.6	Small proline rich-like 1	Sprrl1	3.6	79.6	1419317_x_at
10.6	Hornerin	Hrnr	0.8	13.8	1451613_at
10.6	Keratin complex 1, acidic, gene 15	Krt1-15	2.8	54.3	1422667_at
13.9	Metallothionein 4	Mt4	6.9	138.7	1450645_at
24.3	Troponin C2, fast	Tnnc2	10.2	256.2	1417464_at
26.0	Small proline-rich protein 3	Sprr3	1.7	71.3	1422401_at
27.9	Small proline-rich protein 2A	Sprr2a	1.7	62.4	1450618_a_at
48.5	Keratin complex 2, basic, gene 4	Krt2–4	11.8	561.1	1418735_at
64.0	Repetin	Rptn	0.7	89	1420431_at
256.0	Keratin complex 1, acidic, gene 13	Krt1–13	0.8	351.7	1422454_at
337.8	Loricrin	Lor	0.8	193.8	1448745_s_at

Appendix II. Genes sensitive to 1000 IU diet in MALE lungs.

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			10 IU	1000 IU	
−7.0	Cytochrome P450, family 2. subfamily c, polypeptide 37	Cyp2c37///Cyp2c50///Cyp2c54	11.8	1.4	1419094_at
−4.9	Chemokine (C—C motif) receptor 1	Ccr1	18.2	2.4	1419610_at
−4.3	Adenosine A1 receptor	Adora1	19.3	1.2	1435495_at
−2.8	Gametogenetin binding protein 1	MGI:3055306	39.9	12.7	1433904_at
−2.1	SUMO/sentrin specific protease 6	Senp6	38.5	15.2	1427984_at
−2.0	Arginase type II	Arg2	107.9	54.4	1418847_at
2.0	AT rich interactive domain 4B (Rbp1 like)	Arid4b	21.8	41.8	1431024_a_at
2.0	ATPase, Ca++ transporting, cardiac muscle	Atp2a1	43.9	92.2	1419312_at
2.0	Discoidin domain receptor family, member 2	Ddr2	124.7	244.8	1422738_at
2.0	Heat shock protein 1A	Hspa1a	105.3	233.7	1452388_at
2.0	Immunoglobulin kappa chain variable 8 (V8)	Igk-V8	12	41	1451951_at
2.0	Membrane-spanning 4-domains A6C	Ms4a6c	72.5	113.8	1450234_at
2.0	Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase	Pip5k3	23	43.9	1422994_at
2.0	Prosaposin	Psap	731.5	1681.3	1421813_a_at
2.0	SMC4 structural maintenance of chromosomes	Smc4l1	54.1	108.5	1427275_at
2.0	T-cell receptor beta, joining region	Tcrb-J	15.1	34.1	1427667_s_at
2.0	Twisted gastrulation homolog 1 (Drosophila)	Twsg1	107.5	211.9	1426179_a_at
2.1	A kinase (PRKA) anchor protein 2	Akap2	130.5	260.8	1449168_a_at
2.1	Alpha thalassemia/mental retardation syndrome X-linked	Atrx	20.3	43.4	1420947_at
2.1	Cullin 4A	Cul4a	57.5	122.7	1426060_at
2.1	Cyclin D1	Ccnd1	142.3	282.1	1417419_at
2.1	Gamma-aminobutyric acid (GABA-B) receptor, 1	Gabbr1	11.4	35.9	1422051_a_at
2.1	Histone 1, H2ad	Hist1h2ad	127.4	289.9	1438009_at
2.1	Integrin alpha 3	Itga3	61.6	135.3	1460305_at
2.1	Nuclear receptor co-repressor 1	Ncor1	91.5	245.3	1423201_at
2.1	Phosphate cytidylyltransferase 1	Pcyt1a	46.8	98.6	1421957_a_at
2.1	Splicing factor 3b, subunit 1	Sf3b1	262.7	621.3	1418562_at
2.3	adenosine deaminase	Ada	51.4	131.8	1417976_at
2.3	B-cell leukemia/lymphoma 6	Bcl6	60.1	117.5	1450381_a_at
2.3	Caspase 3, apoptosis related cysteine protease	Casp3	27.9	63.3	1426165_a_at
2.3	CEA-related cell adhesion molecule 1	Ceacam1	17.6	42.8	1427711_a_at
2.3	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	Ddx6	46.5	119.1	1424598_at
2.3	DNA methyltransferase 3A	Dnmt3a	12.3	34.6	1423065_at
2.3	Histocompatibility 2, T region locus 24	H2-T24	34.2	62.6	1422160_at
2.3	Myosin, light polypeptide 3	Myl3	33.5	158.7	1428266_at
2.3	Retinoblastoma binding protein 4	Rbbp4	331.7	731.1	1454791_a_at
2.5	Creatine kinase, muscle	Ckm	94.4	259.9	1417614_at
2.5	High density lipoprotein (HDL) binding protein	Hdlbp	24.2	58.1	1415988_at
2.5	Interleukin 3 receptor, alpha chain	Il3ra	26.3	61	1419712_at
2.5	PRP19/PSO4 homolog (S. cerevisiae)	Prp19	52.8	183.7	1460633_at
2.5	Ring finger protein 184	Rnf184	197.5	584.4	1429109_at
2.6	Albumin 1	Alb1	27.7	89.5	1425260_at
2.6	Avian erythroblastosis virus E-26 oncogene related	Erg	17.2	49.9	1425370_a_at

Appendix II – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			10 IU	1000 IU	
2.6	Fusion, derived from t(12;16) malignant liposarcoma	Fus	33.9	88.5	1455831_at
2.6	*N*-Deacetylase/*N*-sulfotransferase 1	Ndst1	28.5	75.9	1422044_at
2.6	Protein tyrosine phosphatase, non-receptor type 2	Ptpn2	16	47.1	1425197_at
2.6	RNA binding motif protein 5	Rbm5	42.9	97.5	1456262_at
2.6	Tripartite motif-containing 44	Trim44	17.4	48.9	1421869_at
2.6	Vesicle-associated membrane protein	Vapb	149.1	417.6	1423152_at
2.8	Aspartate-beta-hydroxylase	Asph	2	25.2	1425274_at
2.8	E74-like factor 2	Elf2	19.1	48.9	1428045_a_at
2.8	Heat shock protein 1B	Hspa1b	233.4	653.2	1427126_at
2.8	Male sterility domain containing 2	Mlstd2	20.5	52.5	1453550_a_at
2.8	Translocated promoter region	Tpr	59.4	158.5	1456112_at
3.0	Mitogen activated protein kinase kinase kinase 3	Map3k3	17.7	54.7	1426687_at
3.0	Pleckstrin homology domain interacting protein	Phip	51.9	166.8	1423432_at
3.0	Zinc finger protein 146	Zfp146	40.2	107.4	1422135_at
3.2	Kallikrein 6	Klk6	5.5	19	1415837_at
3.2	Kidney androgen regulated protein	Kap	31.4	120.3	1415968_a_at
3.2	Protocadherin 12	Pcdh12	12.6	53.6	1450473_at
3.5	Repetin	Rptn	14.2	49.4	1420431_at
4.0	Large tumor suppressor 2	Lats2	5.2	28.4	1419679_at
4.3	Nucleoplasmin 3	Npm3	29.7	134.8	1423522_at
4.3	Tropomyosin 1, alpha	Tpm1	5.3	31.5	1456623_at
4.6	SRY-box containing gene 5	Sox5	4.6	35.5	1432189_a_at
4.6	Troponin T3, skeletal, fast	Tnnt3	34.9	164.8	1450118_a_at
4.9	Myosin light chain, phosphorylatable, fast skeletal muscle	Mylpf	35.8	179.1	1448371_at
5.3	Max protein	Max	28.6	264.1	1423501_at
5.7	Major urinary protein 1///major urinary protein 2	Mup1/2	87.1	448.1	1420465_s_at
6.5	Defensin beta 4	Defb4	4.6	50.4	1419600_at
6.5	Troponin I, skeletal, fast 2	Tnni2	45.3	247.6	1416889_at
8.6	Loricrin	Lor	18	111.3	1420183_at
12.1	Lectin, galactose binding, soluble 7	Lgals7	2.8	55.7	1422308_a_at
13.0	Growth hormone	Gh	1.8	51.6	1460613_x_at
19.7	Actin, alpha 1, skeletal muscle	Acta1	42.8	808.4	1427735_a_at
19.7	Myosin, heavy polypeptide 1, skeletal muscle, adult	Myh1	9.1	178.5	1427520_a_at
19.7	Small proline-rich protein 3	Sprr3	3.4	64.5	1422401_at
21.1	Metallothionein 4	Mt4	3.7	108.5	1450645_at
22.6	SLIT-ROBO Rho GTPase activating protein 1	Srgap1	0.7	14.6	1431719_a_at
39.4	Kidney androgen regulated protein	Kap	1.5	87.8	1415969_s_at
59.7	Troponin C2, fast	Tnnc2	4.5	361	1417464_at
147.0	Myosin, heavy polypeptide 8, skeletal muscle, perinatal	Myh8	0.6	73.2	1426650_at
168.9	Keratin complex 2, basic, gene 4	Krt2–4	3.9	593.4	1418735_at
222.9	Keratin complex 1, acidic, gene 13	Krt1–13	2.2	363.3	1422454_at

Appendix III. Genes sensitive to 35 IU diet in female lungs.

Fold Δ			Signal		
	Gene title	Gene symbol	< 10 IU	35 IU	Probe set ID
− 157.6	Cytochrome P450, family 3, subfamily a, polypeptide 41	Cyp3a41	35.9	0.1	1419704_at
− 52.0	T-cell receptor gamma, variable 6	Tcrg-V4	9.3	0.2	1452558_at
− 36.8	Uncoupling protein 1 (mitochondrial, proton carrier)	Ucp1	73.5	2.1	1418197_at
− 34.3	Serine (or cysteine) proteinase inhibitor, clade A, member 3K	Serpina3k	74.7	2.2	1423866_at
− 29.9	Syndecan 4	Sdc4	1586.2	39.6	1417654_at
− 18.4	Albumin 1	Alb1	699.9	34.5	1425260_at
− 18.4	Forkhead box K1	Foxk1	26.8	0.9	1451752_at
− 16.0	Major urinary protein 1///major urinary protein 2	Mup1/2	2173.2	123.4	1420465_s_at
− 16.0	Myosin, heavy polypeptide 6, cardiac muscle, alpha	Myh6	22.6	0.6	1417729_at
− 14.9	Serine (or cysteine) proteinase inhibitor, clade A, member 1b	Serpina1b///Serpina1d///Serpina1e	137.3	9.1	1418282_x_at
− 13.9	CLIP associating protein 1	Clasp1	24.8	2	1427353_at
− 13.0	Apolipoprotein A–I	Apoa1	56.5	2.1	1419232_a_at
− 12.1	Proteasome 26S subunit, non-ATPase, 11	Psmd11	28.7	2.9	1456104_at
− 12.1	Sulfotransferase family 2A, member 2	Sult2a2	23.5	1.5	1419528_at
− 10.6	Carbamoyl-phosphate synthetase 1	Cps1	33.2	1.8	1455540_at
− 10.6	Group specific component	Gc	71.8	6.6	1426547_at
− 9.2	Phenylalanine hydroxylase	Pah	42.8	2.8	1454638_a_at
− 8.6	Actin, beta, cytoplasmic	Actb	1247	117.6	1419734_at
− 8.6	Transthyretin	Ttr	93.7	7.5	1451580_a_at
− 8.0	Apolipoprotein A-II	Apoa2	244.9	28.1	1417950_a_at
− 8.0	Implantation serine protease 2	Isp2	29.8	6.7	1449805_at
− 8.0	Serine (or cysteine) proteinase inhibitor, clade A, member 1a	Serpina1a	190.1	14.5	1448680_at
− 7.5	RAB27A, member RAS oncogene family	Rab27a	38.6	4.6	1425285_a_at
− 7.5	Sema domain, immunoglobulin domain (Ig)3A	Sema3a	20.3	1.9	1420417_at
− 7.0	Apolipoprotein C-III	Apoc3	58.9	9.3	1418278_at
− 6.5	Myeloid/lymphoid or mixed lineage-leukemia translocation	Mllt1	20.8	2.6	1421060_at
− 6.1	Aldo-keto reductase family 1, member C6	Akr1c6	30.6	3.8	1417085_at
− 6.1	Major urinary protein 5	Mup5	27.5	3	1426166_at
− 6.1	Plasminogen	Plg	32.5	4.2	1416729_at
− 6.1	Prolactin like protein I	MGI:1914250	11.4	1.9	1420746_at
− 6.1	SAC3 domain containing 1	Sac3d1	15.2	2.7	1417036_at
− 5.3	Arylacetamide deacetylase (esterase)	Aadac	19.9	4.6	1448813_at
− 5.3	Guanine nucleotide binding protein, beta 1	Gnb1	1664.7	363.5	1454696_at
− 4.9	Coronin, actin binding protein 1C	Coro1c	38.5	7.6	1419911_at
− 4.6	AT rich interactive domain 5A (Mrf1 like)	Arid5a	38.7	2.1	1451340_at
− 4.6	Pentatricopeptide repeat domain 1	Ptcd1	58.9	13.6	1416391_at
− 4.6	Purinergic receptor P2X, ligand-gated ion channel, 7	P2rx7	37.5	10	1422218_at
− 4.6	Superoxide dismutase 1, soluble	Sod1	3545.5	841.1	1451124_at
− 4.6	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase	B4galt1	1056.4	220.4	1418014_a_at
− 4.3	PRP19/PSO4 homolog (S. cerevisiae)	Prp19	67.2	22.1	1460633_at
− 4.0	4-Hydroxyphenylpyruvic acid dioxygenase	Hpd	47.7	12.3	1424618_at
− 4.0	Gene model 1120, (NCBI)	Gm1120	105.1	31.3	1456218_at
− 4.0	GLI-Kruppel family member GLI3	Gli3	23.1	4.9	1450525_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
− 4.0	Mannose receptor, C type 2	Mrc2	37	9	1421044_at
− 4.0	Max protein	Max	73.2	17.6	1423501_at
− 4.0	Tubulin, beta 5	Tubb5	782.9	180.2	1455719_at
− 3.7	Actin, alpha 1, skeletal muscle	Acta1	76.4	7	1427735_a_at
− 3.7	Alpha-2-HS-glycoprotein	Ahsg	31.8	4.8	1455093_a_at
− 3.7	Heat shock protein 1	Hspb1	138.1	33	1427853_a_at
− 3.7	Nuclear receptor subfamily 2, group C, member 2	Nr2c2	46.5	13.9	1451569_at
− 3.7	Polymerase I and transcript release factor	Ptrf	73.6	15.7	1421431_at
− 3.5	ADP-ribosylation factor 3	Arf3	45.3	12.4	1421789_s_at
− 3.5	Nucleoplasmin 3	Npm3	63.6	19.1	1423522_at
− 3.5	Ras association (RalGDS/AF-6) domain family 3	Rassf3	75	24.8	1417015_at
− 3.2	B-cell leukemia/lymphoma 6	Bcl6	54.7	18.4	1450381_a_at
− 3.2	Glioblastoma amplified sequence	Gbas	458.2	123.6	1419484_a_at
− 3.2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein . . .	Kdelr2	1857.9	622.8	1417204_at
− 3.2	Retinoblastoma binding protein 4	Rbbp4	339.6	91.5	1454791_a_at
− 3.2	Similar to Cyp2c40 protein	LOC433247	39.5	10.4	1423244_at
− 3.2	Splicing factor, arginine/serine-rich 2 (SC-35)	Sfrs2	412.4	121.6	1452439_s_at
− 3.0	Thyroid hormone responsive SPOT14 homolog (Rattus)	Thrsp	179.6	57.9	1424737_at
− 3.0	Topoisomerase (DNA) II beta	Top2b	40.9	12.8	1448458_at
− 2.8	Inositol hexaphosphate kinase 1	Ihpk1	125.9	44.2	1422968_at
− 2.8	Myosin, heavy polypeptide 9, non-muscle	Myh9	2934.1	937.9	1420172_at
− 2.6	Alpha 1 microglobulin/bikunin	Ambp	26.5	9.9	1416649_at
− 2.6	CAP, adenylate cyclase-associated protein 1	Cap1	125.4	42.5	1417461_at
− 2.6	Glycoprotein 38	Gp38	1881.6	639.1	1419309_at
− 2.6	Golgi SNAP receptor complex member 1	Gosr1	25.6	9.5	1448256_at
− 2.6	Growth hormone	Gh	23.8	10.1	1460310_a_at
− 2.6	Hemopexin	Hpxn	64	21.6	1423944_at
− 2.6	SUMO1/sentrin specific protease 1	Senp1	37	14.4	1451319_at
− 2.5	AF4/FMR2 family, member 1	Aff1	37.1	13.6	1425640_at
− 2.5	Bcl2-like 1	Bcl2l1	253.5	91.4	1420887_a_at
− 2.5	Endothelin converting enzyme 1	Ece1	326	116.1	1434177_at
− 2.5	Heterogeneous nuclear ribonucleoprotein A/B	Hnrpab	1683.9	653.8	1426114_at
− 2.5	Hus1 homolog (S. pombe)	Hus1	41.3	12.3	1425366_a_at
− 2.5	Nuclear receptor subfamily 6, group A, member 1	Nr6a1	27.5	13.4	1421516_at
− 2.5	Serine (or cysteine) proteinase inhibitor, clade C,member 1	Serpinc1	57.7	23.4	1417909_at
− 2.5	v-crk sarcoma virus CT10 oncogene homolog	Crkl	46.2	16.7	1421954_at
− 2.3	Adaptor protein complex AP-2, alpha 2 subunit	Ap2a2	404.3	205.9	1435869_s_at
− 2.3	Bradykinin receptor, beta 1	Bdkrb1	31.7	10	1450586_at
− 2.3	Caldesmon 1	Cald1	165.1	66.4	1424770_at
− 2.3	Chromobox homolog 7	Cbx7	63.6	13.2	1420039_s_at
− 2.3	Epithelial stromal interaction 1 (breast)	Epsti1	28.7	13.6	1432235_at
− 2.3	GATA zinc finger domain containing 1	Gatad1	244.7	136.3	1452085_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
− 2.3	Hypoxia up-regulated 1	Hyou1	229.2	215.4	1423291_s_at
− 2.3	Pregnancy zone protein	Pzp	52.3	19.4	1417246_at
− 2.3	Prosaposin	Psap	965.8	370.7	1421813_a_at
− 2.3	Rad and gem related GTP binding protein 1	Rem1	35.4	18.4	1450158_at
− 2.3	Serine hydrolase-like	Serhl	63.3	40.5	1434145_s_at
− 2.3	Thyroid transcription factor 1	Titf1	101.4	38.1	1422346_at
− 2.3	Tubulin, beta 2	Tubb2	21.8	11.5	1427838_at
− 2.1	Aldehyde dehydrogenase 2, mitochondrial	Aldh2	110.7	55.6	1434987_at
− 2.1	Caspase 9	Casp9	50.9	20.9	1426125_a_at
− 2.1	Gap junction membrane channel protein alpha 1	Gja1	102.5	62.5	1415801_at
− 2.1	Histone 1, H2ad	Hist1h2ad	253.6	172.4	1438009_at
− 2.1	Non-catalytic region of tyrosine kinase adaptor protein 2	Nck2	37.4	13.6	1416797_at
− 2.1	Protease inhibitor 16	Pi16	62.5	22	1453839_a_at
− 2.1	Ring finger protein 184	Rnf184	225.5	90.5	1429109_at
− 2.1	SET translocation	Set	798.9	476.6	1426853_at
− 2.1	Spleen tyrosine kinase	Syk	31.2	17.4	1425797_a_at
− 2.1	SRY-box containing gene 4	Sox4	98.8	44.2	1419157_at
− 2.1	Stomatin	Stom	114.8	47.6	1419099_x_at
− 2.0	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	Pfkfb3	64.7	40.4	1456676_a_at
− 2.0	Adaptor-related protein complex 2, beta 1 subunit	Ap2b1	361.5	132.9	1452292_at
− 2.0	ATPase, Ca++ transporting, cardiac muscle	Atp2a1	23.7	11.4	1419312_at
− 2.0	CASP8 and FADD-like apoptosis regulator	Cflar	96.2	54.3	1425686_at
− 2.0	Claudin 18	Cldn18	602.9	281.8	1425445_a_at
− 2.0	Fructose bisphosphatase 1	Fbp1	60.7	26.1	1448470_at
− 2.0	Kruppel-like factor 6	Klf6	128.2	57.4	1427742_a_at
− 2.0	Lecithin-retinol acyltransferase	Lrat	89.7	44.7	1421345_at
− 2.0	Neurotensin receptor 2	Ntsr2	16.6	10.8	1417151_a_at
− 2.0	Prostaglandin E synthase 3 (cytosolic)	Ptges3	1081.2	645	1460221_at
− 2.0	Sestrin 2	Sesn2	73.7	36	1451599_at
− 2.0	Tripartite motif protein 8	Trim8	60.2	28.4	1438679_at
− 2.0	Vesicle-associated membrane protein	Vapb	129	96.2	1423152_at
− 2.0	Zinc finger protein 36, C3H type-like 2	Zfp36l2	40.9	12.8	1451034_at
2.0	Acetyl-coenzyme A acetyltransferase 2	Acat2	402.5	816.1	1435630_s_at
2.0	Adenylate cyclase 7	Adcy7	268.2	500.9	1456307_s_at
2.0	Aldehyde dehydrogenase 9, subfamily A1	Aldh9a1	243.4	604.7	1436689_a_at
2.0	Aldehyde dehydrogenase family 1, subfamily A7	Aldh1a7	764.5	1703.6	1418601_at
2.0	AMP deaminase 3	Ampd3	126.3	286.6	1422573_at
2.0	Angiogenic factor with G patch and FHA domains 1	Aggf1	138.9	280.9	1425354_a_at
2.0	Angiopoietin-like 4	Angptl4	245.3	490.7	1417130_s_at
2.0	ATPase, H + transporting, V1 subunit D	Atp6v1d	237.1	533	1438993_a_at
2.0	BCL2/adenovirus E1B 19 kDa-interacting protein 1	Bnip3	434.2	815.3	1422470_at
2.0	Bone morphogenetic protein receptor, type 1A	Bmpr1a	204.7	483.5	1425492_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
2.0	Breast cancer anti-estrogen resistance 3	Bcar3	211.6	427.5	1415936_at
2.0	cAMP-regulated phosphoprotein 19	Arpp19	239.1	449.3	1422608_at
2.0	Carboxypeptidase E	Cpe	116.7	202.1	1415949_at
2.0	CCR4-NOT transcription complex, subunit 4	Cnot4	87.6	183.4	1436645_a_at
2.0	CCR4-NOT transcription complex, subunit 6	Cnot6	429.3	852.9	1426682_at
2.0	Cereblon	Crbn	96.6	167.9	1423095_s_at
2.0	Chondroitin sulfate GalNAcT-2	MGI:1926002	332.9	613.5	1424431_at
2.0	Choroidermia	Chm	93.3	219.8	1448712_at
2.0	Cleavage and polyadenylation specificity factor 3	Cpsf3	307.6	581.5	1437852_x_at
2.0	CREBBP/EP300 inhibitory protein 1	Cri1	274.3	611.4	1416614_at
2.0	Cytoplasmic polyadenylation element binding protein 4	Cpeb4	202.9	454	1420618_at
2.0	DEAH (Asp-Glu-Ala-His) box polypeptide 30	Dhx30	16.8	37.9	1453251_at
2.0	Dehydrogenase/reductase (SDR family) member 8	Dhrs8	1108.6	2231.8	1434642_at
2.0	DEP domain containing 6	Depdc6	127.3	253.6	1451348_at
2.0	Der1-like domain family, member 1	Derl1	311.7	636.2	1437723_s_at
2.0	Desmoplakin	Dsp	164.8	341.7	1435493_at
2.0	DnaJ (Hsp40) homolog, subfamily A, member 2	Dnaja2	81.8	178.4	1417182_at
2.0	Dynactin 6	Dctn6	225.5	434.9	1416499_a_at
2.0	Dynamin 1-like	Dnm1l	254.6	513.7	1428086_at
2.0	E74-like factor 5	Elf5	156.2	250.4	1419555_at
2.0	Elongation factor RNA polymerase II 2	Ell2	70.6	151	1450744_at
2.0	Establishment of cohesion 1 homolog 1 (S. cerevisiae)	Esco1	131.6	285.5	1424324_at
2.0	Eukaryotic translation initiation factor 3, subunit 2 (beta)	Eif3s2	563.5	1143.6	1448264_a_at
2.0	Eukaryotic translation initiation factor 5B	Eif5b	176	367.1	1434604_at
2.0	farnesyl diphosphate farnesyl transferase 1	Fdft1	164.9	339.8	1438322_x_at
2.0	Fas (TNF receptor superfamily member)	Fas	167.4	554.6	1460251_at
2.0	Folliculin	Flcn	106.9	233.3	1438167_x_at
2.0	Furin (paired basic amino acid cleaving enzyme)	Furin	72.4	137.4	1418518_at
2.0	G kinase anchoring protein 1	Gkap1	184.6	349.6	1417594_at
2.0	G protein-coupled receptor associated sorting protein 1	Gprasp1	184.9	354.7	1424455_at
2.0	G protein-coupled receptor, family C, group 5, member B	Gprc5b	41.9	82.5	1424613_at
2.0	Germ cell-less homolog (Drosophila)	Gcl	116.2	206	1417113_at
2.0	Golgi phosphoprotein 3	Golph3	182.5	362.1	1450394_at
2.0	Granzyme A	Gzma	296.4	588.9	1417898_a_at
2.0	GTP binding protein 4	Gtpbp4	129.4	251.1	1450873_at
2.0	Guanine nucleotide binding protein (G protein), gamma 10	Gng10	278.3	500.5	1450649_at
2.0	H2A histone family, member Z///similar to H2A histone family, member Z	H2afz	94.1	235.8	1456032_x_at
2.0	Helicase with zinc finger domain	Helz	93.8	137	1427469_at
2.0	Hepatitis B virus x interacting protein	Hbxip	270.3	512.4	1436152_a_at
2.0	Heterogeneous nuclear ribonucleoprotein L-like	Hnrpll	182	343.6	1427144_at
2.0	HIV TAT specific factor 1	Htatsf1	227.1	437.1	1454760_at
2.0	Hydroxyacyl-coenzyme A dehydrogenase beta subunit	Hadhb	287	687.8	1437172_x_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
2.0	Hyperparathyroidism 2 homolog (human)	Hrpt2	99.1	188.8	1427971_at
2.0	Immediate early response 3 interacting protein 1	Ier3ip1	115.1	246.1	1417424_at
2.0	Influenza virus NS1A binding protein	Ivns1abp	293.6	578.3	1450084_s_at
2.0	Insulin induced gene 2	Insig2	82.3	177	1417980_a_at
2.0	Integral membrane protein 2A	Itm2a	135.8	395.9	1423608_at
2.0	Inter alpha-trypsin inhibitor, heavy chain 4	Itih4	266.7	639.3	1431808_a_at
2.0	Itchy	Itch	152.9	313.7	1415769_at
2.0	Kinectin 1	Ktn1	240.6	598.3	1455434_a_at
2.0	Kinesin family member 5B	Kif5b	235.2	497	1418429_at
2.0	Lactotransferrin	Ltf	194.3	387.2	1450009_at
2.0	Laminin B1 subunit 1	Lamb1-1	272.1	532.5	1424114_s_at
2.0	Leucine rich repeat (in FLII) interacting protein 1	Lrrfip1	439.2	832	1433842_at
2.0	Leucine rich repeat containing 35	Lrrc35	180.6	347.4	1454930_at
2.0	Leucine-rich alpha-2-glycoprotein 1	Lrg1	344.5	671.3	1417290_at
2.0	Lipoprotein lipase	Lpl	1013.2	1992.2	1415904_at
2.0	LPS-responsive beige-like anchor	Lrba	196.4	392.7	1449099_at
2.0	Mannosidase, beta A, lysosomal	Manba	98.8	165.2	1450626_at
2.0	Membrane-spanning 4-domains, Subfamily A, member 4D	Ms4a4d	222.2	403.6	1418990_at
2.0	Metadherin	Mtdh	69.1	141.7	1434882_at
2.0	Mitochondrial ribosomal protein L44	Mrpl44	138.1	264.4	1452144_a_at
2.0	Myeloblastosis oncogene	Myb	60.1	110	1450194_a_at
2.0	Nemo like kinase	Nlk	271.1	543.6	1435970_at
2.0	Nuclear receptor coactivator 6 interacting protein	Ncoa6ip	117.2	246.3	1421904_at
2.0	Nuclear receptor subfamily 1, group D, member 2	Nr1d2	657.9	1390.7	1416958_at
2.0	Nucleolar protein 7	Nol7	246.3	520	1420486_at
2.0	Nucleosome assembly protein 1-like 1	Nap1l1	190.2	361.8	1420477_at
2.0	Nudix-type motif 4	Nudt4	450.5	725.9	1418505_at
2.0	O-Acetyltransferase	MGI:2384865	249.4	461.3	1451980_at
2.0	Pellino 1	Peli1	133.6	243.9	1417372_a_at
2.0	Phosphorylase kinase beta	Phkb	149.5	258	1434511_at
2.0	Polymerase (RNA) III (DNA directed) polypeptide K	Polr3k	70.4	128.1	1422752_at
2.0	Potassium channel, subfamily K, member 1	Kcnk1	127.1	205.4	1448690_at
2.0	Profilin 2	Pfn2	221.1	461.1	1418209_a_at
2.0	Prostaglandin-endoperoxide synthase 1	Ptgs1	148.9	327.6	1436448_a_at
2.0	Protein tyrosine phosphatase 4a1	Ptp4a1	281.2	546.4	1455002_at
2.0	Protein tyrosine phosphatase, receptor type, D	Ptprd	198.7	382.7	1429052_at
2.0	RAD23b homolog (S. cerevisiae)	Rad23b	138.7	258.5	1450903_at
2.0	Regenerating islet-derived 3 gamma	Reg3g	411	801.4	1448872_at
2.0	Reticulocalbin 1	Rcn1	140.8	290.3	1417090_at
2.0	Rho GTPase activating protein 5	Arhgap5	195.6	345.7	1450897_at
2.0	Ribosomal protein L5	Rpl5	31.4	79.6	1451077_at
2.0	Ribulose-5-phosphate-3-epimerase	Rpe	69.4	142.2	1416705_at

Appendix III – *Continued*

Fold Δ			Signal		
	Gene title	Gene symbol	< 10 IU	35 IU	Probe set ID
2.0	Ring finger protein 2	Rnf2	150	242.5	1424873_at
2.0	RIO kinase 3 (yeast)	Riok3	410.3	729.9	1415747_s_at
2.0	RNA binding motif protein 7	Rbm7	321.8	679	1451237_s_at
2.0	Sarcolipin	Sln	271.1	466.3	1420884_at
2.0	Sec1 family domain containing 1	Scfd1	121.3	289.1	1428335_a_at
2.0	SEC14-like 4 (S. cerevisiae)	Sec14l4	130.1	269.4	1424676_s_at
2.0	Short coiled-coil protein	Scoc	93.7	158.9	1416267_at
2.0	Similar to KAT protein	LOC226654	30.9	105.4	1456169_at
2.0	Sjogren syndrome antigen B	Ssb	51.8	96.7	1416423_x_at
2.0	Sjogren syndrome antigen B	Ssb	61.1	131.6	1416422_a_at
2.0	Spermatid perinuclear RNA binding protein	Strbp	70.5	117.2	1452061_s_at
2.0	Sphingosine-1-phosphate phosphatase 1	Sgpp1	22.2	76.7	1420821_at
2.0	Splicing factor 3a, subunit 2	Sf3a2	202.7	425.4	1455546_s_at
2.0	Splicing factor, arginine/serine-rich 11	Sfrs11	124.8	235.6	1427269_at
2.0	Splicing factor, arginine/serine-rich 12	Sfrs12	134.2	260.5	1427134_at
2.0	Splicing factor, arginine/serine-rich 3 (SRp20)	Sfrs3	283.5	545.2	1454993_a_at
2.0	Sulfatase modifying factor 1	Sumf1	59.3	128	1424603_at
2.0	Sulfotransferase family 1A, phenol-preferring, member 1	Sult1a1	593.8	995.7	1427345_a_at
2.0	Thioredoxin domain containing 9	Txndc9	280.8	647.9	1436951_x_at
2.0	Thioredoxin-like 1	Txnl1	78.5	156.4	1435235_at
2.0	THO complex 1	Thoc1	68.3	135.3	1424641_a_at
2.0	Thyroid hormone receptor interactor 11	Trip11	226.1	472.2	1427407_s_at
2.0	Torsin A interacting protein 2	Tor1aip2	164.8	279.2	1435526_at
2.0	Transcriptional regulator, SIN3B (yeast)	Sin3b	151.2	266.8	1455039_a_at
2.0	Transducer of ERBB2, 2	Tob2	159.9	289.7	1448666_s_at
2.0	Transducer of ErbB-2.1	Tob1	235.5	330.7	1423176_at
2.0	Transducin-like enhancer of split 4	Tle4	81.7	190.2	1450853_at
2.0	Transforming growth factor, beta receptor I	Tgfbr1	227.6	502	1420895_at
2.0	Translocase of outer mitochondrial membrane 70 hom	Tomm70a	103.4	199.3	1426675_at
2.0	Transmembrane emp24 protein transport domain …	Tmed5	278.5	589.7	1424574_at
2.0	Tubby like protein 4	Tulp4	268	504.8	1448548_at
2.0	Tubby like protein 4	Tulp4	80.6	146.3	1434585_at
2.0	Ubiquilin 2	Ubqln2	137.9	239.3	1450021_at
2.0	Ubiquitin protein ligase E3A	Ube3a	522.8	769.2	1416680_at
2.0	Ubiquitin-activating enzyme E1C	Ube1c	119.8	300.6	1435164_s_at
2.0	WW domain-containing oxidoreductase	Wwox	32.3	75	1431960_at
2.0	Zinc finger and BTB domain containing 20	Zbtb20	328	543.1	1438443_at
2.0	Zinc finger proliferation 1	Zipro1	71.4	136.1	1449732_at
2.0	Zinc finger protein 110	Zfp110	144	271.9	1437236_a_at
2.0	Zinc finger, DHHC domain containing 9	Zdhhc9	44.6	83.5	1454787_at
2.0	Zinc finger, RAN-binding domain containing 1	Zranb1	77.8	185.5	1415712_at
2.1	Amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 13 (human)	Als2cr13	217.1	447.1	1434010_at

Appendix III – *Continued*

Fold Δ			Signal		
	Gene title	Gene symbol	< 10 IU	35 IU	Probe set ID
2.1	Carnitine deficiency-associated gene	Cdv1	136.7	298.3	1419286_s_at
2.1	Cell cycle progression 1	Ccpg1	184.5	343.7	1424420_at
2.1	Chloride channel 3	Clcn3	258.3	323.2	1438366_x_at
2.1	Coactosin-like 1 (Dictyostelium)	Cotl1	235	499.8	1436838_x_at
2.1	Coiled-coil-helix-coiled-coil-helix domain containing 6	Chchd6	72.3	233.8	1438659_x_at
2.1	Cyclin-dependent kinase inhibitor 1A (P21)	Cdkn1a	297.2	708.5	1424638_at
2.1	DEAH (Asp-Glu-Ala-His) box polypeptide 9	Dhx9	452.1	859	1451770_s_at
2.1	Desmoglein 2	Dsg2	52.2	149.6	1439476_at
2.1	DnaJ (Hsp40) homolog, subfamily B, member 9	Dnajb9	372	791	1417191_at
2.1	DnaJ (Hsp40) homolog, subfamily C, member 14	Dnajc14	119.5	281.6	1437546_at
2.1	Down syndrome critical region gene 1-like 1	Dscr1l1	197.2	425.9	1421425_a_at
2.1	F-box only protein 22	Fbxo22	91.5	246.6	1426593_a_at
2.1	FERM domain containing 4B	Frmd4b	238.6	541.4	1438169_a_at
2.1	FUS interacting protein (serine-arginine rich) 1	Fusip1	114.6	243.6	1423982_at
2.1	G protein-coupled receptor 155	Gpr155	168.6	361.3	1452353_at
2.1	Heterogeneous nuclear ribonucleoprotein R	Hnrpr	295.7	553.2	1427129_a_at
2.1	High mobility group nucleosomal binding domain 3	Hmgn3	100.5	262.3	1434875_a_at
2.1	High mobility group nucleosomal binding domain 3	Hmgn3	77.7	181.9	1431777_a_at
2.1	Homeodomain interacting protein kinase 3	Hipk3	623.3	1341.9	1426819_at
2.1	Interferon-induced protein with tetratricopeptide repeats 1	Ifit1	117.5	245.9	1450783_at
2.1	Leucine-zipper-like transcriptional regulator, 1	Lztr1	97.5	234.9	1455928_x_at
2.1	MAD homolog 4 (Drosophila)	Smad4	459.2	901.7	1422487_at
2.1	Membrane-associated ring finger (C3HC4) 7	marf7	311.5	686.7	1420609_at
2.1	Metallothionein 2	Mt2	191.9	403.2	1428942_at
2.1	Methyl-CpG binding domain protein 1	Mbd1	218.8	492.3	1417968_a_at
2.1	Neogenin	Neo1	105.8	220	1434931_at
2.1	Nucleosome binding protein 1	Nsbp1	123	316.1	1418152_at
2.1	PDZ domain containing RING finger 3	Pdzrn3	99.3	211.5	1416846_a_at
2.1	Phosphodiesterase 9A	Pde9a	99.2	201.9	1449403_at
2.1	Phosphoserine/threonine/tyrosine interaction protein	Styx	75	143	1429710_at
2.1	Protease, serine, 23	Prss23	195.1	423.6	1437671_x_at
2.1	Protein inhibitor of activated STAT 1	Pias1	187.5	415.7	1422581_at
2.1	Protein kinase C, epsilon	Prkce	116.6	247	1452878_at
2.1	Pyruvate dehydrogenase kinase, isoenzyme 1	Pdk1	44.6	91.7	1423747_a_at
2.1	RAN binding protein 2	Ranbp2	274	652.6	1450690_at
2.1	RAN binding protein 9	Ranbp9	212.6	445.5	1456199_x_at
2.1	Catalytic subunit of DNA polymerase zeta RAD54 like	Rev3l	167	372.5	1424632_a_at
2.1	Rhophilin, Rho GTPase binding protein 2	Rhpn2	48.6	108.3	1434628_a_at
2.1	Ribosomal protein L17	Rpl17	35.8	96.7	1453752_at
2.1	Ring finger protein 12	Rnf12	48.8	127.4	1439403_x_at
2.1	RNA binding motif protein 16	Rbm16	39.3	94.5	1455310_at
2.1	RNA polymerase II transcriptional coactivator	Rpo2tc1	70.2	145.5	1422692_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
2.1	RNA, U22 small nucleolar	Rnu22	167.6	380.2	1439399_a_at
2.1	SH3 multiple domains 2	Sh3md2	40.7	88.5	1455149_at
2.1	Single-stranded DNA binding protein 1	Ssbp1	348.2	671.7	1427965_at
2.1	Sirtuin 1 ((silent mating type information regulation 2, homolog) 1 (S. cerevisiae)	Sirt1	165.6	227.1	1418640_at
2.1	Small nuclear ribonucleoprotein polypeptide A'	Snrpa1	99.2	240.5	1417351_a_at
2.1	Sulfotransferase family 1D, member 1	Sult1d1	88.1	162	1448973_at
2.1	TAF13 RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf13	190.8	307.5	1448707_at
2.1	Testis derived transcript 3	Tes3-ps	73.6	153.2	1455054_a_at
2.1	Thioredoxin-like 2	Txnl2	76.7	223.5	1456244_x_at
2.1	Torsin family 3, member A	Tor3a	75	189.3	1421998_at
2.1	Transcription factor 3	Tcf3	69	163.6	1450117_at
2.1	Transformation related protein 53	Trp53inp1	381.4	868.9	1416926_at
2.1	Transient receptor potential cation channel, subfamily M	Trpm7	111.3	236.4	1416799_at
2.1	Translocator of inner mitochondrial membrane 44	Timm44	61.9	162.9	1439371_x_at
2.1	Tyrosine 3-monooxygenase activation protein, beta polypeptide	Ywhab	1254.2	2832.1	1455815_a_at
2.1	Very low density lipoprotein receptor	Vldlr	85.5	149.5	1417900_a_at
2.1	WD repeat domain 57 (U5 snRNP specific)	Wdr57	12.7	53	1452713_a_at
2.1	Zinc finger protein 148	Zfp148	32	75.4	1418381_at
2.1	Zinc finger protein 326	Zfp326	51	138.4	1419181_at
2.1	Zinc finger protein 617	Zfp617	82.5	199.7	1449546_a_at
2.3	Aconitase 1	Aco1	146.5	345.6	1456728_x_at
2.3	Aldehyde dehydrogenase family 1, subfamily A2	Aldh1a2	90.5	155.1	1422789_at
2.3	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	Ace2	54	140	1425102_a_at
2.3	Calpastatin	Cast	213.7	496.3	1451413_at
2.3	Choline phosphotransferase 1	Chpt1	81.4	185.3	1455901_at
2.3	Cofilin 2, muscle	Cfl2	117.9	253.2	1418066_at
2.3	Cytosolic acyl-CoA thioesterase 1	Cte1	170	350.4	1449065_at
2.3	Dendritic cell protein GA17	Ga17	11.5	31.6	1437189_x_at
2.3	DNA-damage-inducible transcript 4	Ddit4	174	262.8	1428306_at
2.3	Dystrobrevin alpha	Dtna	44.9	112.3	1456069_at
2.3	Dystrophin, muscular dystrophy	Dmd	54.3	108.4	1448665_at
2.3	Enabled homolog (Drosophila)	Enah	222.4	577.6	1424800_at
2.3	Eosinophil-associated, ribonuclease A family, member 1	Ear1	138.3	276.7	1421802_at
2.3	Eukaryotic translation initiation factor 3, subunit 1 alpha	Eif3s1	29.6	87.3	1426395_s_at
2.3	FBJ osteosarcoma oncogene	Fos	230	522.1	1423100_at
2.3	Flavin containing monooxygenase 3	Fmo3	337.1	875.3	1449525_at
2.3	Glutathione S-transferase omega 1	Gsto1	287.2	829.3	1456036_x_at
2.3	Hepatoma-derived growth factor	Hdgf	108.7	225.1	1415888_at
2.3	Hypoxia inducible factor 1, alpha subunit	Hif1a	235.8	538.6	1427418_a_at
2.3	Isopentenyl-diphosphate delta isomerase	Idi1	28.5	91.3	1423804_a_at
2.3	Lysosomal acid lipase 1	Lip1	64.1	168.4	1423140_at
2.3	Membrane protein, palmitoylated 7 (MAGUK p55 subfamily member 7)	Mpp7	52.4	130.6	1455179_at

Appendix III – *Continued*

Fold Δ			Signal		
	Gene title	Gene symbol	< 10 IU	35 IU	Probe set ID
2.3	Nuclear DNA binding protein	MGI:1927354	170.1	389.6	1448505_at
2.3	Nuclear factor I/B	Nfib	127.3	249.7	1448288_at
2.3	Peroxisomal biogenesis factor 11a	Pex11a	75.4	145.3	1419365_at
2.3	Succinocarboxamide synthetase	Paics	118.7	290.4	1436298_x_at
2.3	RAP2C, member of RAS oncogene family	Rap2c	98.1	201.8	1460430_at
2.3	Ring finger protein 149	Rnf149	269.8	560.7	1429321_at
2.3	RIO kinase 2 (yeast)	Riok2	27.7	86.7	1436684_a_at
2.3	Serine carboxypeptidase 1	Scpep1	158.2	380.7	1455908_a_at
2.3	Serologically defined colon cancer antigen 33	Sdccag33	98.6	220.1	1427233_at
2.3	Solute carrier family 25 member 5	Slc25a5	82	188.3	1438360_x_at
2.3	Special AT-rich sequence binding protein 1	Satb1	39.5	95.8	1416008_at
2.3	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf9	76.3	167.9	1451509_at
2.3	Telomeric repeat binding factor 2	Terf2	90.5	195.8	1421147_at
2.3	Testis expressed gene 10	Tex10	141.8	326.6	1439464_s_at
2.3	Tripartite motif protein 27	Trim27	125	304.7	1456375_x_at
2.3	Ubiquitin specific protease 1	Usp1	213.6	481.9	1451080_at
2.3	X-linked lymphocyte-regulated 3a	Xlr3a	23.1	81.9	1420357_s_at
2.3	Zinc finger protein 161	Zfp161	145.4	267.3	1437585_x_at
2.3	Zinc finger protein 281	Zfp281	50.5	113.9	1426377_at
2.5	Ankyrin 3, epithelial	Ank3	176.7	854.5	1452124_at
2.5	Chondroadherin	Chad	131.7	400.9	1420569_at
2.5	Crystallin, zeta	Cryz	41.6	107	1438610_a_at
2.5	Cystic fibrosis transmembrane conductance regulator	Cftr	32.7	73.7	1420579_s_at
2.5	Endothelial differentiation 2	Edg2	95.2	173.7	1417143_at
2.5	Enhancer of yellow 2 homolog (Drosophila)	Eny2	56.7	156.1	1429411_a_at
2.5	F-box only protein 3	Fbxo3	57.3	149.1	1455047_at
2.5	Fibromodulin	Fmod	41.2	87.6	1456084_x_at
2.5	Forkhead box P1	Foxp1	427.1	991	1435222_at
2.5	Gap junction membrane channel protein beta 6	Gjb6	23.5	82.3	1448397_at
2.5	Glycerophosphodiester phosphodiesterase domain containing 1	Gdpd1	112.7	292.1	1424076_at
2.5	Inhibitor of growth family, member 3	Ing3	46.3	112.6	1422806_x_at
2.5	Kruppel-like factor 9	Klf9	930.7	2505.5	1456341_a_at
2.5	Lanosterol synthase	Lss	29.8	89.2	1426913_at
2.5	Macrophage receptor with collagenous structure	Marco	48.9	203.2	1449498_at
2.5	Mucin 5, subtype B, tracheobronchial	Muc5b	256.4	508.6	1427626_at
2.5	Neurobeachin	Nbea	155	364.4	1452251_at
2.5	Opioid growth factor receptor-like 1	Ogfrl1	37.3	111.3	1424413_at
2.5	Pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	100.5	195.1	1417273_at
2.5	RAD21 homolog (S. pombe)	Rad21	64.3	200.5	1455938_x_at
2.5	RUN and SH3 domain containing 1	Rusc1	76.4	187.1	1434743_x_at
2.5	Sideroflexin 1	Sfxn1	143.5	363.9	1417559_at
2.5	Solute carrier family 6 member 14	Slc6a14	73.3	189.1	1420504_at

Appendix III – *Continued*

Fold Δ	Gene title	Gene symbol	Signal		Probe set ID
			< 10 IU	35 IU	
2.5	Thioredoxin-like 5	Txnl5	194.6	596	1439184_s_at
2.5	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase	B3galt3	45.2	112.5	1418736_at
2.5	Vanin 1	Vnn1	85.3	254.7	1418486_at
2.5	Yamaguchi sarcoma viral (v-yes) oncogene homolog 1	Yes1	47.6	118.9	1449090_a_at
2.5	YTH domain family 3	Ythdf3	128.8	339	1426840_at
2.6	cutC copper transporter homolog (E.coli)	Cutc	22.1	64.6	1418473_at
2.6	glutamate receptor, ionotropic, *N*-methyl D-asparate-associated protein 1 (glutamate binding)	Grina	157.2	379.1	1436297_a_at
2.6	Lipin 1	Lpin1	78.6	225.9	1418288_at
2.6	Necdin	Ndn	59.6	173.3	1435382_at
2.6	Period homolog 2 (Drosophila)	Per2	64.5	173.3	1417602_at
2.6	Protocadherin alpha subfamil C1	Pcdha4	45	141	1420798_s_at
2.6	Ring-box 1	Rbx1	15.7	35.1	1438623_x_at
2.6	Sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A	Sema7a	249.6	480.5	1459903_at
2.8	Dopa decarboxylase	Ddc	21	61.5	1426215_at
2.8	Kelch-like 2, Mayven (Drosophila)	Klhl2	60.4	199.6	1426978_at
2.8	Sestrin 1	Sesn1	41.2	208.1	1438931_s_at
2.8	Sorbin and SH3 domain containing 1	Sorbs1	136.5	370.9	1436737_a_at
2.8	SRY-box containing gene 2	Sox2	76.7	178.3	1416967_at
2.8	Transforming growth factor alpha	Tgfa	58.8	150.4	1421943_at
3.0	PHD finger protein 17	Phf17	42.2	161.7	1452179_at
3.0	Schlafen 4	Slfn4	30.2	73.5	1427102_at
3.0	Ubiquitin specific protease 2	Usp2	40.1	126.3	1417169_at
3.0	Wee 1 homolog (S. pombe)	Wee1	49.3	163.4	1416773_at
3.2	CUG triplet repeat, RNA binding protein 2	Cugbp2	124.6	364.8	1423895_a_at
3.2	Leucine zipper protein 5	Luzp5	14	59.3	1417926_at
3.2	Natriuretic peptide precursor type A	Nppa	50.8	159.7	1456062_at
3.5	Calcium/calmodulin-dependent protein kinase II	Camk2b	233.7	845.8	1455869_at
3.7	Plasmacytoma variant translocation 1	Pvt1	6	36.7	1452324_at
4.6	Farnesyltransferase, CAAX box, beta	Fntb	13.5	67.8	1434309_at
4.6	FK506 binding protein 5	Fkbp5	112.1	487.5	1416125_at
4.6	Killer cell lectin-like receptor subfamily C, member 1 / 2	Klrc1 /2	5.3	50.8	1425005_at
6.1	Sema domain, seven thrombospondin repeats	Sema5a	13	81.9	1434776_at
6.5	Eukaryotic translation termination factor 1	Etf1	8.2	48.2	1420023_at
7.0	Proteasome subunit, alpha type 1	Psma1	0.8	15.5	1436769_at
9.2	Gamma-aminobutyric acid (GABA-B) receptor, 1	Gabbr1	9.8	84.7	1437188_at