EFFECT OF OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACIDS ON GENE EXPRESSION

An Undergraduate Research Scholars Thesis
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Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Sophia Valdez, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Faculty Research Advisor prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Effect of Omega-3 and Omega-6 Polyunsaturated Fatty Acids on Gene Expression

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Foods play an essential role in the management of disease; it is becoming increasingly common for individuals to turn to foods as a form of therapy or even prevention of progressive conditions, such as cancer. The biochemical makeup of a particular food can alter gene expression at a cellular level, possibly resulting in the prevention of cellular replication processes that ultimately lead to the unwanted initiation and growth of tumors. This project investigates the effects of both omega-3 (fish oil) and omega-6 (corn oil) polyunsaturated fatty acids in preventing the initiation and progression of colon cancer by gathering statistical trends in the presence of mRNA and miRNA, using samples retrieved from the colon of rats. This is accomplished using a data science programming language R, under R Studio interface environment, that helps the user to efficiently model, manipulate, and visualize expansive genomic data. It is hypothesized that negative regulation of mRNA by miRNA will be shown, by these two molecules essentially having an inverse relationship. Earlier research suggests that omega-3 polyunsaturated fatty acids can alter gene expression in the colon, meaning that they have highly useful chemopreventive properties. It is also hypothesized that the presence of

omega-6 polyunsaturated fatty acids does not assist in the initiation of cancer; but nonetheless, provides no statistically substantial prevention. Upon completion of analysis within the scope of this project, omega-3 polyunsaturated fatty acids and omega-6 polyunsaturated fatty acids cause much differential expression amongst genes in the context of colon cancer. Within this work, solid evidence was found to support the negative regulatory relationship of mRNA and miRNA. It is the goal that, with even further analyses and data integration, conclusions will provide support for the importance of incorporating foods that are contain omega-3 polyunsaturated fatty acids into one’s diet. Common sources of this dietary fat include seafood and leafy vegetables, which are easy additions to an everyday diet, and likely carry a multitude of other health benefits as well.

DEDICATION

To my friends, family, and faculty mentor, who have all supported me throughout this research process. This journey has been so rewarding and was only possible through your encouragement.

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Contributors

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All other analyses conducted for the thesis was completed by the student independently.

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NOMENCLATURE

MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
R	R Studio
mRNA	Messenger Ribonucleic Acid
miRNA	Micro Ribonucleic Acid
PCR	Polymerase Chain Reaction
NCBI	National Center for Biotechnology Information
FC	Fold Change

1. INTRODUCTION

1.1 Background

It has long been acknowledged that the food individuals consume directly affects their health, and not only by means of fitness and overall nutrition. The American Institute for Cancer Research, as well as the World Cancer Research Fund, both assert that anywhere from up to 40% of all cancer cases can be prevented with the use of appropriate diet, exercise, and body weight maintenance [15]. On the contrary, however, some foods are carcinogens, and can encourage the initiation of a tumor within the body.

Polyunsaturated fatty acids (PUFAs) are a common subject of research interest. There's an extensive list of benefits from this dietary fat that have been suggested before, specifically from n-3 PUFAs. Some of those benefits include prevention and treatment of cardiovascular disease, atherosclerosis, and arrhythmias [12]. Omega-3 PUFAs are commonly found in fish oil, seafood, and some nuts and seeds. Omega-6 PUFAs are commonly found in corn oil, vegetable oils, soy, and in general, most processed foods. They are clearly a common staple in the food we eat, and meaningful parts of a many consumer's diets. This work aims to ascertain the benefits of PUFAs in a very distinct way: identifying a connection between the ingestion of PUFAs and the gene expression in intestinal epithelial cells in relation to colon cancer initiation and progression. This research was performed in the context of an animal model. Specifically, rats, with the high conservation of genetic material across mammalian species being reason to apply any findings to human health.

On a chemical level, the only difference between omega-3 and omega-6 PUFAs is the location of the first appearance of double bonds along their long carbon chain. But this small difference makes for a big change with how the body processes and uses them. This shift in location of the double bond changes the entire structure of the molecule. The more double bonds a fatty acid has, the more bent it is. Furthermore, the more double bonds a molecule has, the higher its boiling point will be. These differences in properties result in the body processing these molecules differently, which is why this project aims to discern what kind of physiological differences arise from this structural difference in the two polyunsaturated fatty acids commonly found in the foods we eat every day.

1.2 Source Research

This project utilizes data collected in a study published in 2004 [13]. The aim of that study was to ascertain the notion that omega-3 polyunsaturated fatty acids (PUFAs) had inhibitory effect on the initiation of colon cancer, given that the mechanism of this process has yet to be completely confirmed. In this study, male Sprague Dawley rats were given three different types of dietary fats, two types of carcinogen treatment, as well as two types of dietary fibers. Once the course of treatment was completed, a portion of the colon, the colonic epithelium, was resected. RNA (ribonucleic acid) was isolated from those tissue samples. The RNA was then processed using bioarrays to quantify the expression of both mRNA and miRNA in each one of the samples. This is the origin of the data used in both this original research paper, as well as this project.

The original research from which data was gathered followed a 3 x 2 x 2 experimental design. Two kinds of PUFAs were used: omega-3 PUFAs, omega-6 PUFAs, and omega-9 PUFAs. These were delivered via standard diets of food pellets administered to the rats. In the original research, omega-9 PUFAs were also included as a treatment group. But this factor has been removed to increase statistical strength in the analyses. The next factor in this experimental design in the administration of two possible injections: saline, which has no effect on the body, or AOM, which is a known carcinogen that is commonly used to induce colon cancer in rats for research purposes. Finally, the original work also gave rats two different dietary fibers: cellulose and pectin. Again, however, this factor was agglomerated with the others to increase the strength of the statistical analyses. Therefore, the experimental design of this paper is a simpler 2 x 2 design. These analyses will cover data from the samples of a total of 48 rats.

With the conclusion of their study, Davidson's team concurred that omega-3 PUFAs caused notable difference in the gene expression of cells within the colonic epithelium. This conclusion was not due to the *absence* of either of the other oils; rather, it was due to the presence of fish oil in the treatment. To be clear, neither PUFA seemed to have properties that would exacerbate the cancer progression. It was the presence of fish oil that inhibited the progression of the tumor within the colon.

While this work uses the same data as the original research, the methods by which analyses were conducted are different and novel in several aspects. This invites a new perspective, and at the very least, validation that the findings in the original work still hold true if no new and differing conclusions are reached. Furthermore, this new project considers miRNA data alongside the data used in the original findings; previously, only mRNA data was considered. By introducing an entirely new data set and integrating the analyses of the two types of genomic data, one could get a different perspective that might have more insight on the systems and physiology involved in the chemoprotective action of omega-3 PUFAs, thus creating a reason to reiterate the objectives of the Davidson's original work, without exactly replicating their methods.

1.3 mRNA and miRNA

Within the human body, cell replication is a constant process. It is integral to survival that one’s cells are constantly replicating and replenishing themselves. Within every cell, there are long strands of a biomolecule called deoxyribonucleic acid (DNA). DNA is essentially a biochemical code that decides every aspect of a person’s body and function. This information is sorted into genes, each of which has instructions for the creation of a specific protein. To perform protein synthesis, many other biomolecules and compounds must play a part. In this study, two such molecules (mRNA and miRNA) were the integral source of data, and the basis on which statistical findings were made.

Messenger RNA (mRNA) assists in the process of protein synthesis. DNA is translated into mRNA, and mRNA is then used like an instruction manual to build proteins. Thus, mRNA is expected to be present in the cell when protein synthesis is occurring, further indicating that the cell is growing, changing, and moving towards the process of cell replication. Uncontrolled cell replication commonly leads to cancer – once a cell loses control of the dormant-to-replicating cycle that it sits in, it will replicate again and again, creating tumors. The presence of more mRNA for a particular gene could indicates active gene expression. Some genes, when expressed, initiate, and perpetrate the process of cell replication, or even apoptosis, which is cell death. Thus, it is logical to draw the conclusion that the presence (increased or decreased with respect to its normal levels) of mRNA, in some cases, is an indication of cancer initiation. Of course, to make these findings obsolete, the exact gene being expressed in a specific instance should be investigated regarding its physiological purpose. This would require insight on protein synthesis, as well as any possible regulatory feedback loops, if applicable.

MicroRNA (miRNA) is the second biomolecule of interest. The introduction of miRNA data into the analyses is what makes this research different from Davidson's original work. Molecules of mRNA can help cells control both the amount and kind of proteins made. miRNA will bind to mRNA, and essentially render it inactive [6]. Thus, miRNA is necessary to prevent the overproduction of proteins through this inverse relationship. In terms of relevance to this paper, the high presence of specific miRNA would suggest that the treatment given has chemopreventive properties, because miRNA is a regulator of gene expression, protein synthesis, and consequently, cancer initiation and progression. The integrative analysis of both miRNA and mRNA is what makes this research novel. Furthermore, it is a goal of this project to find evidence of the biological hypothesis that there is, in fact, negative regulation of targeted mRNA by miRNA molecules.

1.4 R Studio

To analyze such large quantities of data, a programming language called R and the interactive user-friendly environment for its application, R-Studio, were used. R is a common data science programming language. R is particularly efficient in terms of data visualization. This is especially the case with the help of external coding "packages" that are free for use. Once downloaded into the program's library, the user can deploy these packages to streamline the statistical analyses and data visualization.

The utilization of R in this project is another means by which the work of the original research team and their data collection can be built upon. Not only does this programming language offer a new perspective and means by which to perform statistical data analyses, it is also done with the addition of the new miRNA data.

1.4.1 Limma Package

With the addition of packages, R can perform specialized tasks. With the addition of the Limma package [21], R can analyze a unique type of imported data known as polymerase chain reaction (PRC) data. This data is organized in the form of a bioarray, a high throughput platform for miRNA detection and quantification. Bioarrays are essentially a canvas of the entire genome, with different focuses tailored to the user's objective. Limma can assist R in handling complex experimental designs, as well as the extreme sizes of these bioarrays.

For mRNA data gathering, Codelink arrays were used. Codelink arrays are a product of the Codelink microarray platform, consists of many species-specific probes that will measure gene expression [14]. There is an R-package, also named Codelink, that is specifically designed to process data obtained by Codelink microarrays. However, when using the Codelink package to upload these data sets, the formatting was not correct. After many attempts, it was decided that Limma would be used instead as an alternative R package that was still capable of statistically modeling and uploading Codelink arrays properly.

2. METHODS

2.1 Treatments

The experimental design that resulted in the datasets used in this research is described in [13]: the animals (rats) were distributed among four treatment groups, Table 1. This is an example of a 2 x 2 x 2 experimental design – with three factors (injection, dietary oil, and dietary fiber). Each factor has two level: three types of PUFAs, two forms of administration, and two forms of fiber. While the original research used a third form of PUFAs, n-9, this treatment group will be disregarded in this research with the goal of conciseness as well as strengthening statistical results. Thus, the dietary fats of important in this work are fish oil and corn oil, which contain n-3 PUFAs and n-6 PUFAs, respectively. These dietary fats were administered via a standard diet of food pellets, which the rats had one week to become accustomed to. After being on a steady diet of their assigned pellets for three weeks, the rats were administered injections of either saline or AOM. Saline in this instance is simply a control group – it has no effect on the prevention or initiation of cancer in the colon, nor anywhere else. However, AOM is a known carcinogen, and will promote the initiation of cancer.

The original data collected included a third factor within the treatment groups: the introduction of different sources of dietary fiber. Cellulose and pectin were the substances used. However, to the increase statistical power of analyses in this study, this fiber factor was agglomerated into the other two factors, creating a simpler 2 x 2 experimental design for this research, seeing as we are maintaining focus on only the PUFAs as independent variables.

Table 1: Experimental Treatment Groups.

Treatment Group	**Dietary Fat**	**Injections**
1	Corn Oil	AOM
2	Corn Oil	Saline
3	Fish Oil	AOM
4	Fish Oil	Saline

Note: Olive oil was also used in the original data collection but given that this research does not take the treatment groups with olive oil into account, these groups are omitted.

A flow diagram inspired by the faculty research advisor of this project was referenced frequently over the course of this project (Figure 1). This map is the framework for how the processing of this project's data was conducted and is a useful reference to guide one's understanding of the procedure in this work.

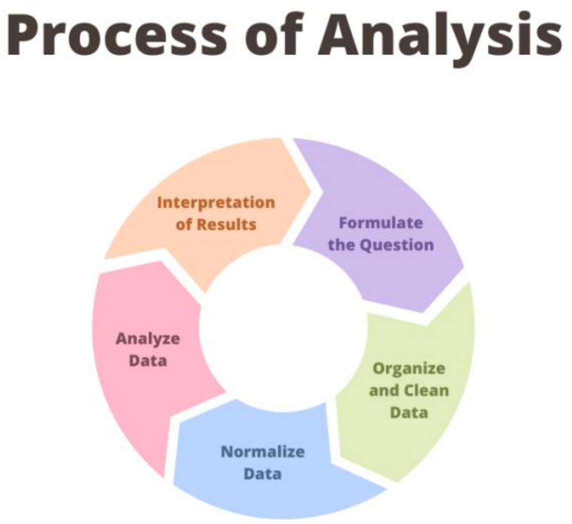

Figure 1: Analyses Flow Diagram

2.2 Types of Arrays

2.2.1 Codelink Arrays

Codelink arrays are a product of the Codelink microarray platform and consist of many species-specific probes that will measure gene expression. This platform is also useful due to its ability to assign quality flags to each gene probe, which proves to be especially helpful during data filtering. While human-specific gene probes weren't used in this data collection for obvious reasons, the fact that many genes are widely conserved between mammalian species such as rats and humans, makes these findings highly applicable to humans as well.

When gathering mRNA data, spot analyses are created, with each spot corresponding to a probe for a particular gene. Codelink arrays can "look" at this visual, and then measure the intensity of a spot, quantify it, and normalize that value across the array of a single rat subject. Thus, if a Codelink array shows us that a particular gene probe has a high normalized intensity, we can consider that gene to be "on", or at least expressed more relative to another array where the intensity is smaller. Therefore, genes with this sort of variability between treatment groups should be acknowledged and investigated concerning their exact physiological function within the body.

2.2.2 PCR Arrays

PCR arrays, which stands for polymerase chain reaction, are a common method of analyzing the expression of miRNA. One might recognize this name in the context of the COVID-19 pandemic when PCR tests were frequently used to identify the presence of the COVID-19 virus by detecting genetic material. The same is done here, but the subject is a rat instead of a virus. As discussed previously, the high rate of conservation of genetic material across mammalian species is why these findings, although gathered within an animal model, are highly applicable to humans. In fact, the mammalian genome contains hundreds of miRNAs, most of which are highly conserved amongst even the most distantly related mammal species [23]. This is why the detectors used in the PCR data collection all begin with “hsa”, which stands for “Homo sapiens”, indicating that the detector is looking for human miRNA within the rats [19].

In this project, low density real time quantitative PCR is used to gather data. In a PCR test, analytic machinery performs cycles of a “search” for miRNA. A total of up to 40 complete cycles will be performed on the sample, piece by piece, by detectors that look for a specific type of miRNA. If no miRNA is found for that detector, then the value given by the PCR test is 40. However, miRNA might be found around, say, the 28th cycle. Then, 28 is the value given in this analysis. The more miRNA that is present, the quicker the PCR test will pick up on it, and the lower the value will be. Therefore, if the PCR test shows lower values for a particular detector, the gene or function in question is being heavily regulated / limited by the high presence of miRNA.

2.3 Filtering

2.3.1 mRNA

Within Codelink arrays, each read of a spot intensity is assigned a quality flag. Many spots will be assigned a G flag to signify that the signal to raise ratio is good; essentially, there is not too much background noise or other intrusions that could compromise the confidence of the data reading. However, not all spots receive this flag. Some are marked with M, which essentially means the read is no good, and the intensity value comes out to a -9999, which is completely negligible data. Other flags, such as L, essentially signal that the signal to noise ratio is just ok; the reading isn’t a super confident one, but it could still be usable alongside other data.

When filtering the arrays in this study, it was decided that any gene probe producing a quality flag other than “G” would be eliminated for analysis. This kind of filtering is done to eliminate abnormalities that could possibly skew data, as well as focus in on probes that would deliver results with more statistical strength and confidence.

This filtering was performed using the Limma software package within R. In each of the 48 arrays, the quality flags, originally recorded as qualitative data, were transformed into quantitative data so that they could be recognized and filtered by Limma. Once this method of labeling and filtering the data had been accomplished, only gene probes made up completely of G flagged intensities across all samples were remaining.

2.3.2 miRNA

With PCR arrays, a wide range of values is possible, mostly ranging from about 20-40 cycles. However, any reading within the range of 35-40 cycles is widely considered in the field as a reading that indicates no presence of miRNA. Thus, values above 35 are essentially indicative of an absence of miRNA. While it is certainly an important observation to note that this target gene is not being regulated, many detectors returned a value of 40 or similar across every single one of the subject rats. To clean up this data, it was decided that if a particular detector had at least five rats return values that were below the range of 35, this detector was kept and analyzed. This means that about 10% of the data for that single detector was viable and considered in our analysis.

This filtering was done pre-transformation of data, which will be discussed in the following section. Filtering was done at this point in data processing for the sake of efficiency. If transformation were to occur after filtering, there would be an excessive amount of data going through the transformation process that would eventually be eliminated anyway. Many detectors, for example, showed values of 40 for every single rat across the board. Given this information, clearly this detector has no significance in this study, as it will not provide a straightforward difference between any of the treatment groups. It's for this reason that filtering, and elimination of some data must take place prior to the transformation of data, even if the count values used for filtering are not as relevant as gene fold expression data.

2.4 Normalization

2.4.1 Median vs. Quantile Normalization

In the Codelink arrays, certain normalization steps have already taken place. One of the most important parameters measured by these arrays, in terms of this study, are the spot intensities. The quantified values of the presence of the spot on the visual analysis of these colon epithelial tissue samples provides an understanding of the presence of mRNA for a particular gene in reference to the other samples.

However, on Codelink arrays, there is both a raw spot intensity and a normalized spot intensity. As one might expect, the raw intensity values are far larger and varied than the normalized intensity values. This method of normalization is known as median normalization, which scales the samples so that they will all have the same median. However, this method of normalization only considers up to the 50th percentile of data.

Alternatively, the normalization method for mRNA used in this study is known as quantile normalization. Quantile normalization has the goal of making the distribution of the values of all arrays the same. It also considers up to the 100th percentile of the data as opposed to only a portion of the data analyzed. This method is performed by firstly ranking each gene in each sample by its magnitude, and then computing the average value of all genes that are within the same rank. Next, the values of all genes within a certain rank are replaced with this average value. Finally, the genes are put back into their original order within the sample [24].

Quantile normalization was performed on both mRNA Codelink arrays. The raw intensity values given by the arrays were used, as opposed to the normalized intensity values generated through median normalization. The process of quantile normalization was performed within R, using the Limma package, and the command “normalizeBetweenArrays” with the argument “method = ‘quantile’”.

2.4.2 Transformation of miRNA PCR Array Data using the $2^{-\Delta CT}$ Method

The data that is produced from PCR is quantified in the form of count data. Essentially, the amount of miRNA present is measured by the count of how many cycles it takes for the PCR detector to detect the presence of miRNA. The values in this data range from roughly 20-40; meaning, it takes anywhere from 20 cycles to 40 cycles to find any miRNA. However, for this data to be more relevant to this study, it must be transformed. By using the $2^{-\Delta CT}$ Method, which will be discussed later, the count data will be transformed into expression data. Expression data will essentially give a value that is proportional to the number of copies found of the miRNA molecule being detected. This form of data is much more helpful for the work being completed in this study and will help to increase statistical strength of the analyses performed.

To complete this task, it was necessary to find the “reference gene”. The reference gene is the gene with which all other genes are compared within PCR data. It is, essentially, the star student whose values are used to normalize all the rest of the values. To find the detector for this, this gene had to be identified. This was accomplished by determining the mean and standard deviation for every one of the detectors. Then, for each detector, the standard deviation was divided by the mean, which in this work was referred to as finding the “% of the Mean”. This value that was generated would be indicative of how far that detectors values deviated from the overall average of its values.

Once this calculation was performed for each of the detectors, they were ranked from lowest value to highest value. The lowest value (meaning that detector had the last amount of variation amongst itself) would then be selected as the reference gene. In this work, that detector was "hsa-miR-320-4373055".

For the next step of transformation, a formula known as the $2^{-\Delta CT}$ Method was used (Equation 1). Using this newfound reference gene alongside the rest of the data, the $2^{-\Delta CT}$ Method will give us the "relative gene expression levels between different samples" [20]. Gene fold expression levels are vital in the thorough analysis of miRNA from its original PCR values, as they give a far better indication of the presence or absence of miRNA.

$$\Delta CT = CT_{(target\ sample)} - CT_{(reference\ sample)}$$

Equation 1. $2^{-\Delta CT}$ Method Formula

Commonly in other literature, the $2^{-\Delta CT}$ Method is not what will be used. Typically, research and analytics will utilize the $2^{-\Delta\Delta CT}$ Method, which is included below (Equation 2). However, the $2^{-\Delta\Delta CT}$ Method requires that there is an untreated sample to accompany every treated sampled, so that the two may be compared. In this project, we do not have a control group of completely untreated rats – though some were administered saline as a control injection, no rats were administered saline, as well as kept from ingesting either of the PUFAs. Thus, the $2^{-\Delta\Delta CT}$ Method cannot be used properly, and the $2^{-\Delta CT}$ Method is implemented instead. This will not negatively impact the analysis or findings in any way. Relative gene fold expression data will still be created and reliable using this method.

$$\Delta CT = \Delta CT_{(target\ sample)} - \Delta CT_{(reference\ sample)}$$

Equation 2. $2^{-\Delta\Delta CT}$ Method Formula

2.5 Analysis

2.5.1 Contrast Groups

Four different contrasts were drawn between treatment groups (Table 2). These contrasts were established by creating contrast matrices in R, as well as using a variety of manipulations of the experimental design. These contrasts were used in some of the final stages of manipulation of this data.

Table 2. Contrast Groups

Contrast Group	**Diet(s)**	**Injection(s)**	**Contrasting Variables**
1	Omega 3 and Omega 6	AOM	Omega 3 vs. Omega 6
2	Omega 3 and Omega 6	Saline	Omega 3 vs. Omega 6
3	Omega 3 and Omega 6	AOM and Saline	AOM vs. Saline

Both mRNA and miRNA data, post-transformation, were then analyzed in R. The command "eBayes" was used to gene probes or detectors, respectively, in order of evidence for differential expression.

Once analysis of data was complete, the command "topTable" was used to generate tables of the most differentially expressed genes within each individual contrast group. These tables showed gene probes or detectors in order according to their P. Value, which is an indication of the likelihood of an observable difference between that probe/detector and the rest of the data.

For Contrast Groups 1 and 2, the raw P. Value was used to determine how many data points would be included. All values less than 0.05 were kept and used in analysis. However, for Contrast Group 3, the Adj. P. Value was used, with any values less than 0.05 being kept.

Once these lists of data were created, then resources such as Codelink and the National Center for Biotechnology Information (also known as NCBI) were used to identify each of the gene probes from the Codelink arrays. For the miRNA PCR arrays, an online microRNA target database called Targetscan [8] was used to identify targets of each of the miRNA detectors.

3. RESULTS

3.1 Normalization

3.1.1 Effects of $2^{-\Delta CT}$ Normalization on miRNA Data

Using the methods mentioned earlier for finding a reference gene in the data to be used in the $2^{-\Delta CT}$ Method procedure, the reference detector selected was “has-miR-320-4373055”. It’s respective values for every rat were used to normalize the rest of the values for each rat. Once this was complete, the $2^{-\Delta CT}$ Method was used to generate a completely new set of miRNA data, now both filtered and normalized. The PCR count data before normalization through this method was relatively similar between treatment groups, with a very small range (Figure 2). However, after completion of this normalization, range drastically increased within this data (Figure 3).

It should be noted that while quantile normalization is visualized here and is a proper step that should be taken in this means of data processing, that was not the case in this project. Data was analyzed without quantile normalization, and thus results might not be as replicable or statistically strong as desired. Barring time constraints, this issue would have been reworked, however due to the limits of this project, this step was foregone. However, the visualization is still present to showcase the effect of quantile normalization, and how it efficiently normalizes data to have strictly equal quantiles.

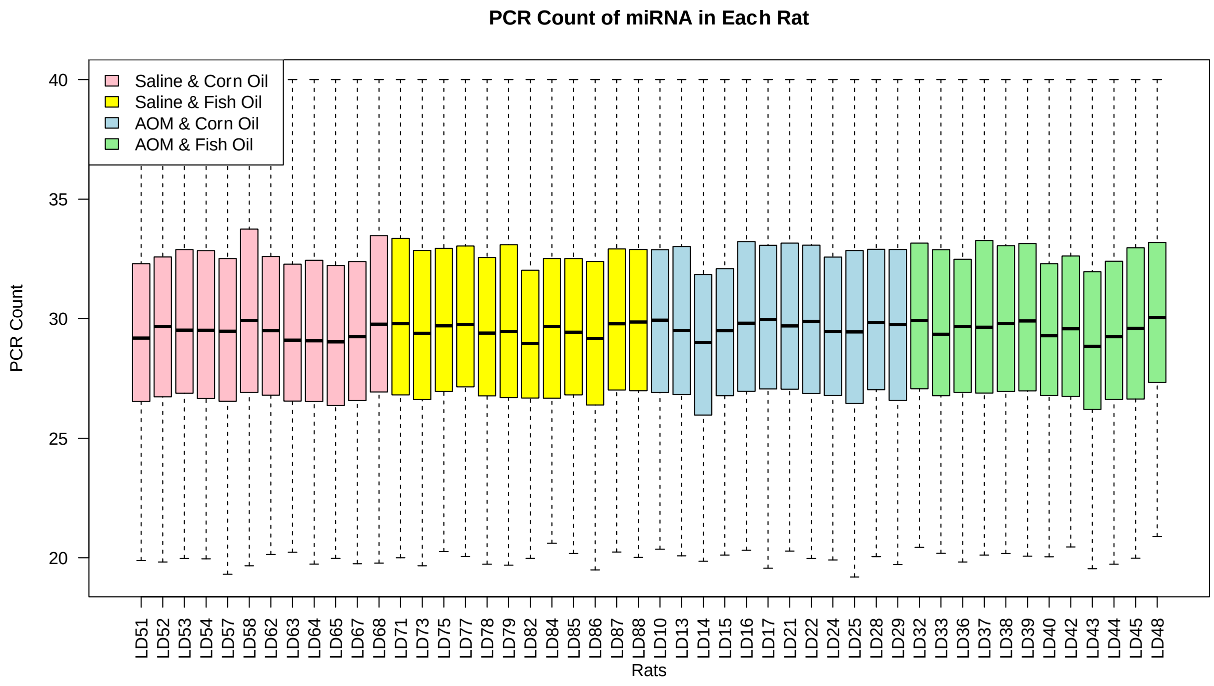

Figure 2: PCR Count of miRNA in Each Rat

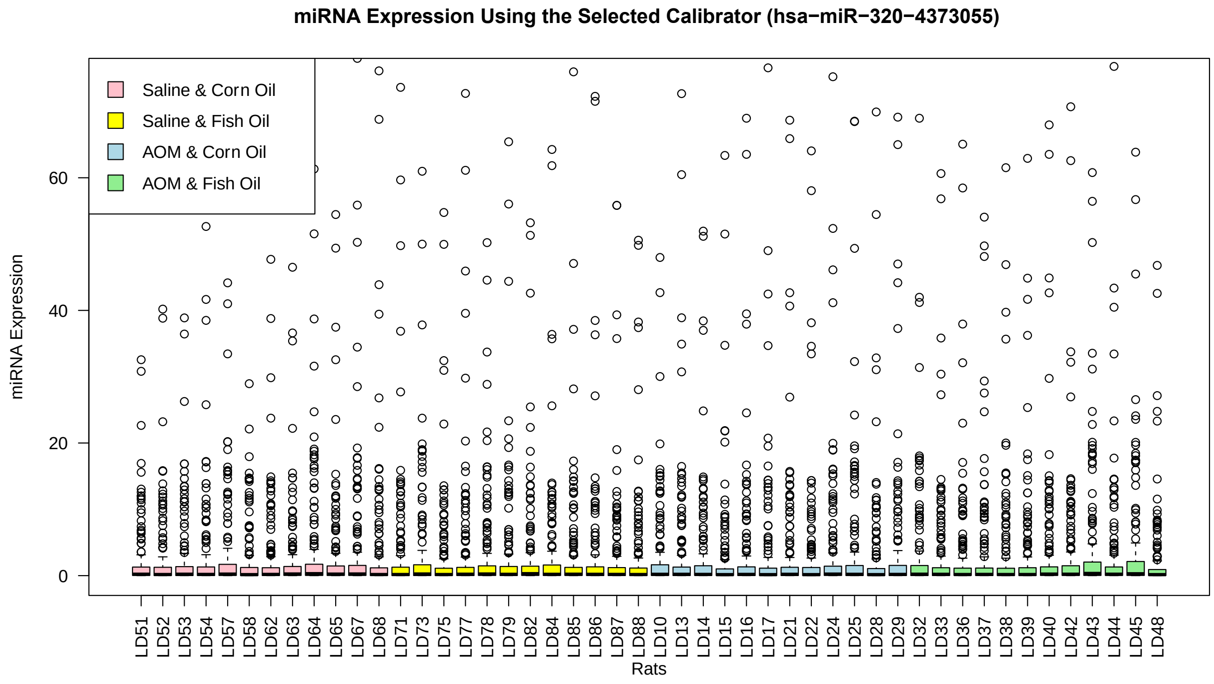

Figure 3: Data Transformed from PCR Counts into miRNA Expression Using the Selected Calibrator (hsa-miR-320-4373055)

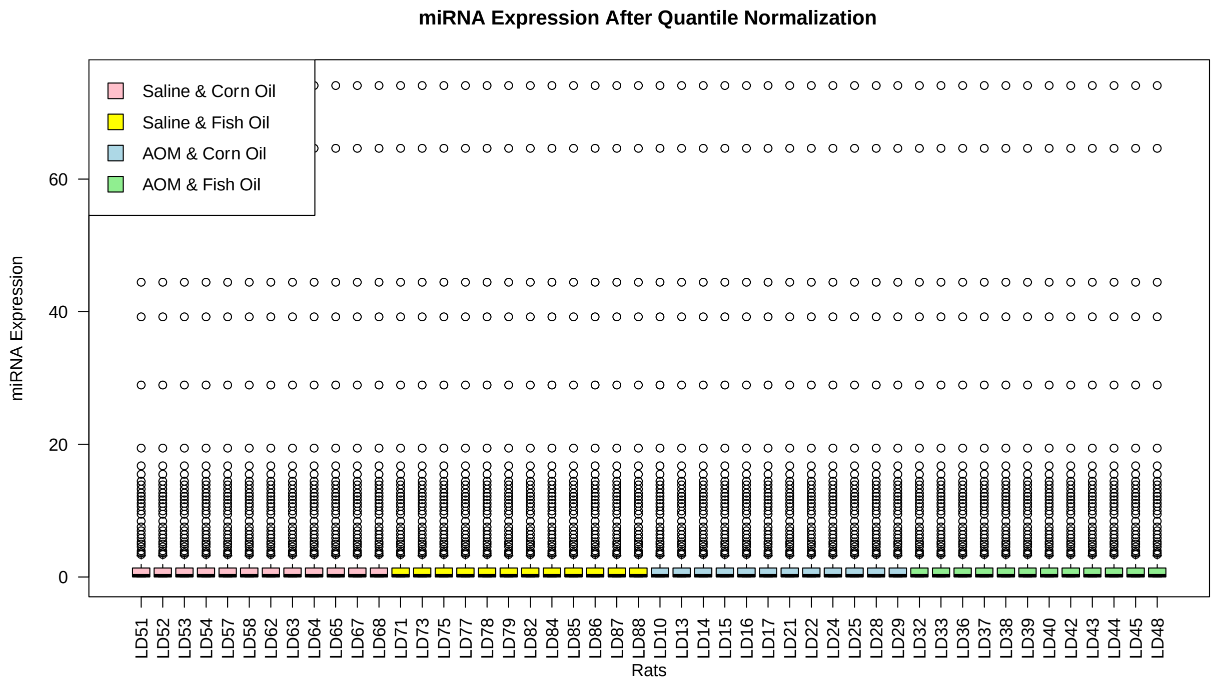

Figure 4: miRNA Expression After Quantile Normalization

3.1.2 Effects of Quantile Normalization in Limma

The procedure of quantile normalization was discussed in 2.4.1. mRNA data was visualized both before and after this process (Figures 5 and 6), and the change to the values is easy to see. The even distribution of values across all treatment groups will not hinder the ability to find differentially expressed genes. Rather, the data is now easier to work with and manipulate with a much smaller range and distribution.

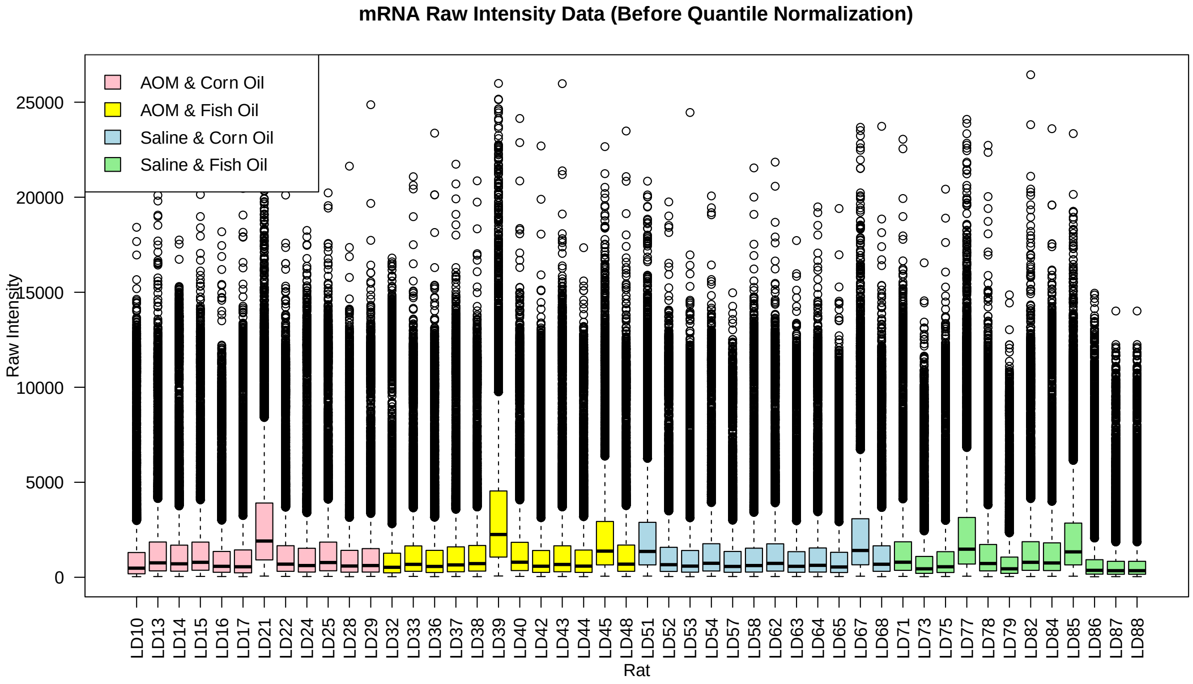

Figure 5: Data Before Quantile Normalization

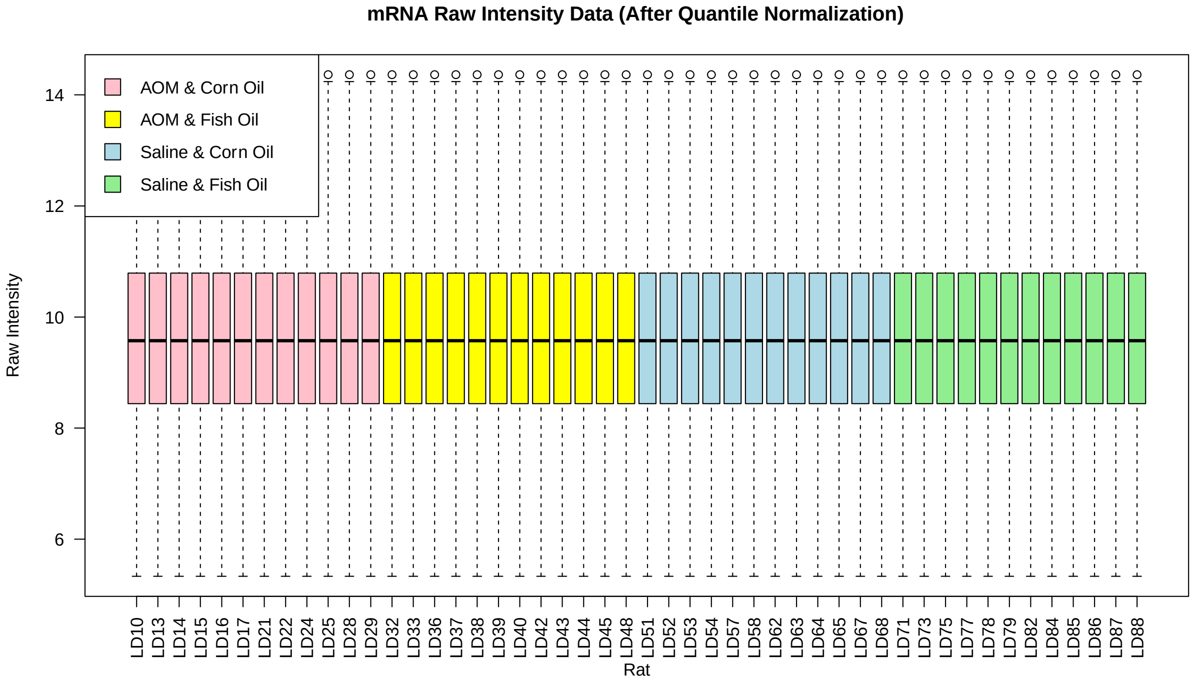

Figure 6: Data After Quantile Normalization

3.2 Analysis

3.2.1 Differentially Expressed Genes

The primary goal of this project was to generate lists of differentially expressed genes from both mRNA and miRNA data, so that these lists could be analyzed, and conclusions could be drawn from these analyses.

It should be noted that, in these analyses, specifically in the mRNA data from Contrast Group 3, the adjusted P. Values were used instead of the raw P. Values. In both instances, genes with either type of P. Value under 0.05 were taken into consideration. This was done because Contrast Group 3 generated so many differentially expressed genes due to the comparison being drawn between a carcinogen and a control group. Thus, a more select, smaller list was used for analysis. However, the full list of differentially expressed genes is included for reference in this paper (Appendix A.3).

3.2.1.1 mRNA

The lists of differentially expressed genes that result from the mRNA data (Appendix A.1, A.2, A.3) are separated based upon the treatments that they specifically contrast.

In Contrast Group 1, the gene with the lowest P. Value from the mRNA, indicating the highest level of differential expression between omega-3 and omega-6 PUFAs, is the androgen-dependent TFPI-regulating protein gene, also known as Adtrp. This gene promotes hydrolase activity [1]. Hydrolase enzymes are necessary for breaking down nutrients, as they use water to cleave chemical bonds [3]. Expression of this gene is higher in the presence of fish oil, or omega-3 PUFAs. The FC (fold change) for this gene is 1.547215229, which means that Adtrp was expressed roughly one and a half times more in the presence of omega-3 PUFAs than in the presence of omega-6 PUFAs. This ratio is important in determining just how much changes in treatment effect the expression of genes. Within Contrast Group 2, which has only saline treatment, the P. Value for this gene is 0.760611834. However, in Contrast Group 3, the P. Value is 0.916963047, which indicates less differential expression.

In Contrast Group 2, the gene with the lowest P. Value from the mRNA, indicating the highest level of differential expression between omega-3 and omega-6 PUFAs, is aquaporin 8, also known as AQP8. This gene is responsible for water channel proteins within the cell [2]. While the connection has not been fully proven, it has been suggested that AQP8 could be a potential marker for cervical cancer, as it seemingly inhibits apoptosis of cells [17]. Expression of this gene is higher in the presence of fish oil, or omega-3 PUFAs. The FC for this gene is 1.853190896.

In Contrast Group 3, the gene with the lowest P. Value from the mRNA, indicating the highest level of differential expression between omega-3 and omega-6 PUFAs, is interferon gamma inducible protein 47, also known as Ifi47. This gene is enables GTPase activity [4]. Expression of this gene is higher in the treatment groups that received AOM injections. The FC for this gene is 1.602167518. In Contrast Group 2, in the presence of saline, this gene had a P. Value of 0.973608313. However, in Contrast Group 1, in the presence of AOM, this gene had a P. Value of 0.144410611, indicating less differential expression in the presence of saline than AOM.

3.2.1.2 miRNA

The lists of differentially expressed miRNA (Appendix A.4, A.5, A.6, A.7, A.8, A.9, A.10, A.11) are separated based upon the treatments that they specifically contrast. Each family of miRNA has hundreds of possible target genes that it could regulate, but for this project, the 20 first results were used for each detector. The methodology for exactly how all these targets are sorted can be found on the Targetscan website and considers important qualities such as level of conservation across genomes [8]. For each Contrast Group, the top target of the miRNA with the lowest raw P. Value was considered and investigated.

The gene of interest from Contrast Group 1, which is the top target for miR-532-3p, is called the zinc finger protein 514 gene, also known as ZNF514. This gene is predicted to be involved in the regulation of transcription by RNA polymerase II [10]. The miRNA that regulates this gene was expressed higher in the presence of fish oil, or omega-3 PUFAs. The FC for this miRNA strand is 1.004876729.

The gene of interest from Contrast Group 2, which is the top target for miR-126-3p, is called the plexin B2 gene, also known as PLXNB2. This gene is part of the B class of plexins, which are transmembrane receptors that are involved in cell migration in response to semaphorins [7]. Semaphorins are extracellular signaling proteins that are needed for the growth and maintenance of organs and tissues [11]. Furthermore, connections have been made between high expression of PLXNB2 and the “proliferation, invasion, and poor prognosis of tumors” [18]. The miRNA that regulates this gene was expressed higher in the presence of fish oil, or omega-3 PUFAs. The FC for this miRNA strand is 1.427650223. In Contrast Group 1, the average expression of this gene in the presence of AOM was 12.70029716, which is higher expression than many other genes in the same conditions.

The gene of interest from Contrast Group 3, which is the top target for miR-146-5p, is called the immunoglobulin superfamily member 1 gene, also called IGSF1. While this gene has a broad array of responsibilities, many of which are thought to participate regulation of interactions between cells, this gene is also involved in the regulation of transcription [5]. The miRNA that regulates this gene was expressed higher in treatment groups that received AOM injections. The FC for this miRNA strand is 0.905059121.

3.2.2 Overlaps

3.2.2.1 mRNA

All the differentially expressed mRNA genes for each contrast performed were compared as well (Figure 7). To discern contrast groups in this diagram, refer to Table 2. It should be noted that while this visualization of the three contrast groups is including all genes from all groups with raw P. Values less than 0.05, in the actual analysis of data in this project, the Adjusted P. Values were instead used for Contrast Group 3, at the same cutoff of 0.05.

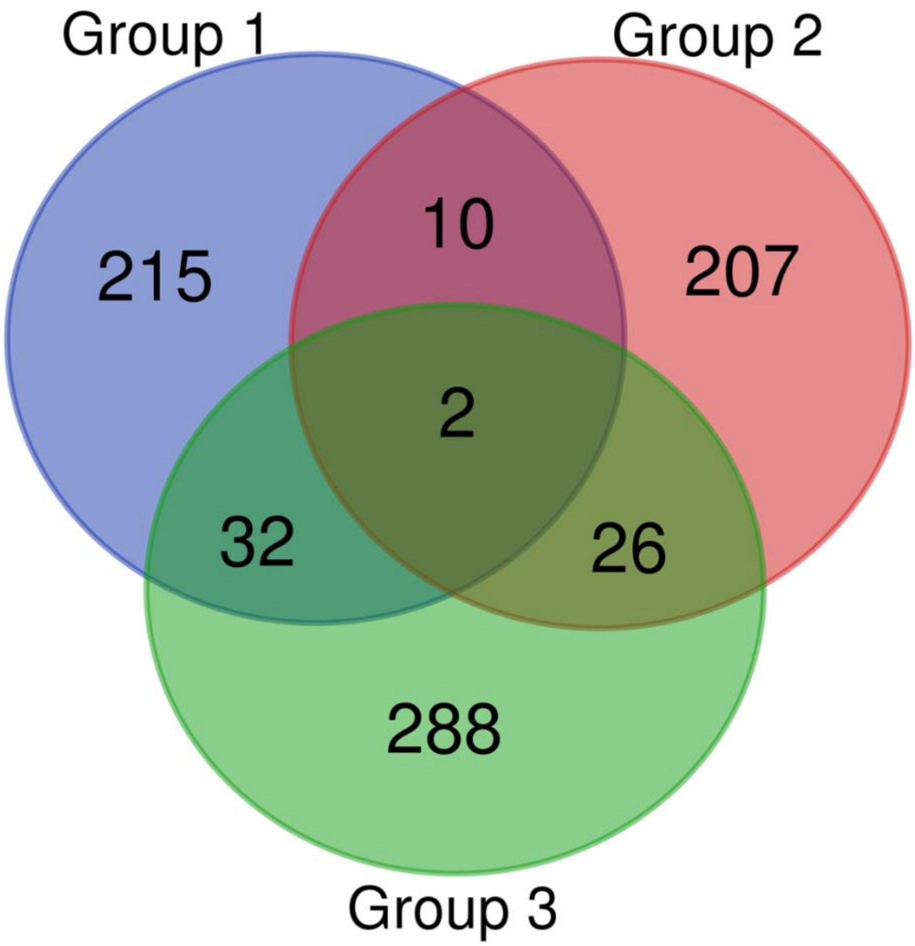

Figure 7: Overlaps of mRNA Gene Probes Between Lists of Differentially Expressed Genes

3.2.2.2 miRNA

All the differentially expressed mRNA genes for each contrast performed were compared as well (Figure 8). To discern contrast groups in this diagram, refer to Table 2.

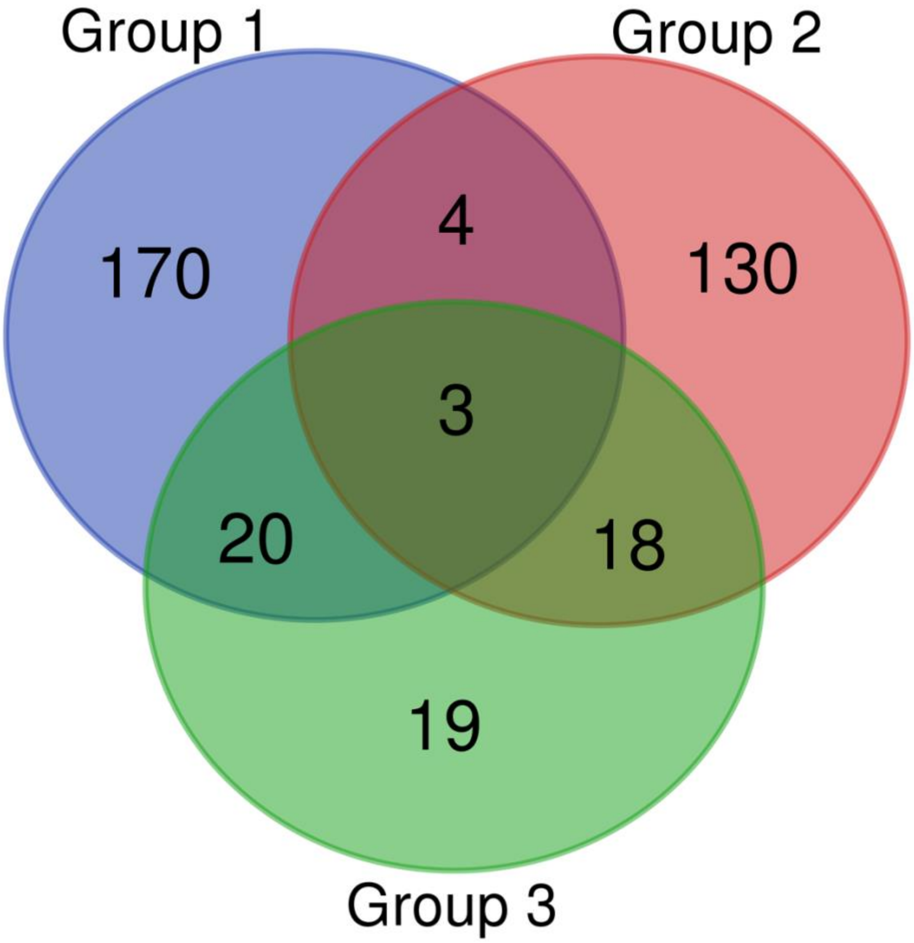

Figure 8: Overlaps of miRNA Target Genes Between Lists of Differentially Expressed Genes

3.2.2.3 mRNA and miRNA

For each individual contrast, the mRNA data and miRNA data were compared to see if any genes were differentially expressed in both sets of data. In Contrast Group 2, the gene SDC2 was differentially expressed in both mRNA and miRNA. In the miRNA data, expression of the miRNA that targets this gene was increased in the Fish Oil treatment groups. In the mRNA data, expression of this gene was decreased in the Fish Oil treatment group. Therefore, it can be asserted that expression of the SDC2 gene is lower in the presence of omega-3 PUFAs than in the presence of omega-6 PUFAs, validated by both the mRNA and miRNA data.

SDC2, also known as syndecan 2, encodes for a transmembrane (type I) heparan sulfate proteoglycan. This is an integral transmembrane protein that is involved in an assortment of cell functions such as cell binding and cell signaling [9]. Interestingly, according to a recent study, SDC2 expression increases dramatically in colorectal cancer cell tissue, and over expression of this gene plays a carcinogenic role in these cells [16].

4. CONCLUSION

4.1 Contrasts

4.1.1 Omega-3 vs. Omega-6 in the Presence of AOM

Adtrp was found to have higher expression in the presence of omega-3 PUFAs. Given that this gene plays a necessary part in breaking down nutrients, it can be concluded that fish oil helped to increase the activity of hydrolysis in the cell, which also indicates a production of energy. Given this information, it could be implied based on the mRNA data that omega-3 PUFAs help to increase the efficiency of nutrient usage within the body.

The miRNA that is known to target and regulate ZNF514 saw increased expression in the presence of omega-3 PUFAs. Given that this gene regulates transcription, it seems that the administration of fish oil led to a deregulation of transcription in the body. Given the presence of AOM in this contrast group, it is not surprising that transcription became unregulated, as this often leads to the initiation and progression of tumors. However, it is important to note that this deregulation of transcription is more pronounced in the presence of omega-3 PUFAs.

Given both products of this contrast analysis, it could be argued that omega-3 PUFAs contributes to the formation of tumors in the colon – this is due to both the deregulation of transcription and increased hydrolysis of nutrients in the cell, both of which are activities that could precede and promote cell division in a certain context.

4.1.2 Omega-3 vs. Omega-6 in the Presence of Saline

AQP8 was expressed more in the presence of omega-3 PUFAs than in the presence of omega-6 PUFAs. If Dr. Weibo Li’s suggestion that AQP8 has been shown to inhibit apoptosis is treated as a solid finding, then this brings the conclusion that omega-3 PUFAs have an inhibiting effect on apoptosis of cells, which is a common precursor the progression of tumors, as well as chemo-resistance [22].

PLXNB2 was highly regulated in the presence of omega-3 PUFAs. Given PLXNB2's close relationship to the progression of tumors, it could be hypothesized using this contrast that the presence of fish oil helps to regulate and inhibit tumors. However, further insight would be needed to confirm these findings, such as data regarding protein synthesis.

4.1.3 AOM vs. Saline with both Omega-3 and Omega-6 Diets

With Ifi47, a gene that enables GTPase activity, it was found that in the presence of AOM, expression increased. This is a logical conclusion, given that increase in GTPase would also lead to increases in other cell activities that come after GTP hydrolysis, such as cell cycle progression, and thus the replication and multiplication of new cells.

It was stated earlier that IGSF1 is involved in the regulation of transcription. The miRNA that has been known to regulate this gene saw higher expression in the presence of AOM rather than saline. This means that the presence of AOM leads, in this situation, to a deregulation of transcription. This is to be expected, given that deregulation of transcription can further lead to unregulated cell growth and replication, all likely brought on by AOM, a carcinogen.

The conclusions from this contrast are exactly what is expected; in these samples, AOM seems to induce increased replication of cells, and the quickening of the cell cycle.

4.2 SDC2 Gene

The SDC2 gene was found to be differentially expressed in Contrast Group 2 from both the miRNA and mRNA data. In the Fish Oil treatment groups, the presence of the miRNA that regulates this gene was increased in contrast to treatment groups with Corn Oil. From the miRNA, it was found that the miRNA that targets SDC2 was expressly highly in the presence of omega-3 PUFAs, which means this gene was highly regulated in these conditions. Therefore, it can be asserted this gene is more regulated in the presence of omega-3 PUFAs than in the presence of omega-6 PUFAs.

When considering the biological hypothesis that mRNA is a molecule that is regulated by miRNA, this analysis is a strong piece of evidence for the confirmation of this hypothesis. It is apparent that there is in fact negative relation of target mRNA by the presence of miRNA, which leads to a strong indication of significance from these findings.

Following the claim that SDC2 expression is increased dramatically in cancer cells in the colon [16], the evidence is strong that omega-3 PUFAs, in this situation, could play a role in helping to regulate SDC2 expression. Granted, this would need further, more diverse data to make any further claims.

This gene was differentially expressed in the context of Contrast Group 2, which only has treatment groups that were administered saline. So, while this is strong evidence to support the biological hypothesis of negative regulation of mRNA by miRNA, it is difficult assert these findings as universal because it has occurred within the expected control group.

4.3 Final Thoughts

With the data available in this project, there is much to be expanded upon. And yet, there is much that we do not have. If it was possible to obtain protein concentration data, it would help greatly in supplementing this work. Due to the complications provided by regulatory pathways and feedback looks within the body, it is not possible to use this data alone to make conclusions about whether either of the PUFAs studied influences the initiation, progression, or prevention of tumorous growths within the colon. However, data on actual protein synthesis from these genes of interest would shine a brighter light on the possibility of making these conclusions. For example, the size of the tumors in the colon was used in conjunction with the mRNA data in the original source research [13].

Furthermore, the genes that were looked at in each group are merely a small sample size. More thorough investigation would require combing through each gene that is differentially expressed within all the generated lists, as well as comparing the possible physiological overlap with each other. Figures 7 and 8 are fantastic visualizations of this; the genes that are differentially expressed, albeit under different contrast groups, would be worth looking into.

Due to time constraints, these goals could not be reached. Nonetheless, they seem promising given the varied results of this project in terms of actual affects, positive or not, of either omega-3 or omega-6 PUFAs. While omega-3 PUFAs have been shown to have a myriad of health benefits [12], additional context to the work done here could possibly lead to a different conclusion.

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APPENDIX: LISTS OF DIFFERENTIALLY EXPRESSED GENES

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE12548	RGD1305679_predicted	0.6296739	1.54721523	0.00001001	0.1386206
GE1173087		-0.8572548	0.55200192	0.00070011	0.9742401
GE14232	Cxcl9	-0.6626500	0.63171687	0.00116183	0.9742401
GE14459		-0.5231625	0.69584481	0.00125329	0.9742401
GE12678		0.7283847	1.65678305	0.00129126	0.9742401
GE14148		-0.4152023	0.74991433	0.00153281	0.9742401
GE1189245		-0.5192562	0.69773146	0.00175079	0.9742401
GE1157676		-0.4795894	0.71718171	0.00180491	0.9742401
GE16570		-0.3755550	0.77080883	0.00185699	0.9742401
GE13401		-0.5757564	0.67093439	0.00189397	0.9742401
GE14546	Ca1_predicted	0.6324304	1.55017426	0.00206539	0.9742401
GE1146578	Hsd3b1	1.2757767	2.42129137	0.00219604	0.9742401
GE1232740		-0.6747898	0.62642348	0.00220233	0.9742401
GE1167182	RT1-S3	-0.4504601	0.73180942	0.00233065	0.9742401
GE21084		0.4851923	1.39977243	0.00300305	0.9742401
GE13910		-0.4843018	0.71484294	0.00312677	0.9742401
GE18958		-0.8726352	0.54614835	0.00396889	0.9742401
GE20029	Sftpd	-0.4827644	0.71560511	0.00410042	0.9742401
GE1293470		-0.3685865	0.77454099	0.00453047	0.9742401
GE13720	Fcgr3a	-0.6118606	0.65435226	0.00538196	0.9742401
GE17841		-0.2784620	0.82446948	0.00560889	0.9742401
GE1274789	Pola2	0.3882530	1.30880757	0.00620986	0.9742401
GE1210352	Sdc3	-0.5386524	0.68841365	0.00647213	0.9742401
GE1301794		-0.3119855	0.80553239	0.00678095	0.9742401
GE18258	Irf7_predicted	-0.6356061	0.64367034	0.00689758	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1208725	Eps8l1_predicted	-0.3014010	0.81146400	0.00691349	0.9742401
GE1277339		-0.3707975	0.77335488	0.00721357	0.9742401
GE18137		-0.3737919	0.77175140	0.00725732	0.9742401
GE22080		-0.6139459	0.65340713	0.00730719	0.9742401
GE1205988		-0.6178633	0.65163532	0.00735879	0.9742401
GE1124484	RGD1304869_predicted	0.3846626	1.30555442	0.00739550	0.9742401
GE1292545	Cte1	0.8048560	1.74697141	0.00748614	0.9742401
GE1195736		-0.4309499	0.74177323	0.00749720	0.9742401
GE14128		-0.3957329	0.76010314	0.00758596	0.9742401
GE13630		-0.6157609	0.65258562	0.00765205	0.9742401
GE1271897		-0.5925598	0.66316520	0.00783914	0.9742401
GE20599		0.4155010	1.33376177	0.00796778	0.9742401
GE1209287		-0.4067868	0.75430150	0.00817583	0.9742401
GE20844		-0.5674469	0.67480993	0.00818335	0.9742401
GE15572	RGD1306494_predicted	-0.3713840	0.77304055	0.00856043	0.9742401
GE1260210		-0.3653614	0.77627439	0.00870212	0.9742401
GE1249754		-0.4562043	0.72890146	0.00897330	0.9742401
GE1286280		-0.3548040	0.78197587	0.00898923	0.9742401
GE21196	Cyb5m	0.2673202	1.20357012	0.00913601	0.9742401
GE21364	Slc22a3	0.3720367	1.29417858	0.00925839	0.9742401
GE15937		-0.5395483	0.68798628	0.00926175	0.9742401
GE12799		-0.2702578	0.82917137	0.00930182	0.9742401
GE1169283	Impa2	0.2414775	1.18220277	0.00943385	0.9742401
GE17886		0.3886531	1.30917059	0.00982861	0.9742401
GE21219		-0.4613998	0.72628123	0.01003383	0.9742401
GE1125357		-0.9316408	0.52426175	0.01023935	0.9742401
GE1128496	Rapgef1	-0.2890797	0.81842397	0.01047018	0.9742401
GE21169		-0.3076956	0.80793123	0.01073800	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1137315		-0.2681675	0.83037361	0.01075180	0.9742401
GE1174391		-0.3964100	0.75974648	0.01106506	0.9742401
GE1296739		-0.3859654	0.76526674	0.01127745	0.9742401
GE1101872		-0.2567217	0.83698768	0.01145274	0.9742401
GE1258989		-0.2850165	0.82073222	0.01146834	0.9742401
GE14591		-0.2091709	0.86503421	0.01165247	0.9742401
GE1146964		-0.5854605	0.66643658	0.01190794	0.9742401
GE1304692	Mcpt9	-0.7918545	0.57760114	0.01194754	0.9742401
GE17289		-0.4120914	0.75153313	0.01230752	0.9742401
GE1130823		-0.669951	0.62852803	0.01296208	0.9742401
GE1281379		-0.4585703	0.72770705	0.01324045	0.9742401
GE1252674		-0.369879	0.7738474	0.01339663	0.9742401
GE17533		-0.24548	0.8435351	0.01352401	0.9742401
GE1206360		0.5286543	1.44258297	0.01403017	0.9742401
GE12637		-0.2747528	0.82659194	0.01411106	0.9742401
GE1243776		-0.2406159	0.84638391	0.01411578	0.9742401
GE1177186		-0.3162691	0.80314418	0.01504863	0.9742401
GE17109		-0.282266	0.82229844	0.01527884	0.9742401
GE1289949		0.3910901	1.31138391	0.01527913	0.9742401
GE1138603		-0.3853019	0.76561877	0.01529439	0.9742401
GE1153672	Pola1	0.4999401	1.41415485	0.0154181	0.9742401
GE1117141		-0.3535396	0.78266151	0.0155683	0.9742401
GE1291001		-0.5842833	0.6669806	0.01631206	0.9742401
GE13281		-0.5753741	0.67111221	0.01657007	0.9742401
GE1127164		-0.3289239	0.79613009	0.01674596	0.9742401
GE1288491		-0.3009535	0.81171574	0.01678773	0.9742401
GE1184652	Lpd	0.6129632	1.52939727	0.01687593	0.9742401
GE19511	P2ry6	-0.3118101	0.80563033	0.01719758	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE18586	Akap2_predicted	-0.4805251	0.71671671	0.01739293	0.9742401
GE1111946		0.4864439	1.40098732	0.01765533	0.9742401
GE18078		-0.3074158	0.80808794	0.01781962	0.9742401
GE1171261		-0.2287602	0.85336793	0.01783278	0.9742401
GE1152228		-0.3455049	0.78703249	0.01784321	0.9742401
GE1275326		-0.280144	0.82350882	0.01869863	0.9742401
GE17049		-0.3335131	0.79360163	0.01873707	0.9742401
GE1109245		-0.2492956	0.84130709	0.01878099	0.9742401
GE1160411		-0.3883145	0.76402169	0.01880714	0.9742401
GE1143269		-0.3173649	0.80253438	0.01916143	0.9742401
GE1187109		0.565213	1.47960594	0.01969536	0.9742401
GE16215		-0.3711913	0.77314381	0.02023398	0.9742401
GE19205		-0.2153273	0.86135073	0.02088787	0.9742401
GE1272576	Scd2	0.3949755	1.31492043	0.02115741	0.9742401
GE21394		-0.4865865	0.71371179	0.02182018	0.9742401
GE20140	Scgb1a1	-0.5702445	0.67350264	0.02210569	0.9742401
GE18264		-0.4396091	0.73733436	0.02223321	0.9742401
GE15917		-0.296068	0.81446917	0.02239818	0.9742401
GE1203014		-0.3917732	0.76219223	0.02243981	0.9742401
GE14706		-0.2666401	0.8312532	0.02250378	0.9742401
GE1242201		0.2801047	1.214283	0.0229159	0.9742401
GE1296536		-0.2248422	0.85568861	0.02301681	0.9742401
GE1137111		-0.2622616	0.83377984	0.02375303	0.9742401
GE22252		-0.5934091	0.66277491	0.02394586	0.9742401
GE1254289		-0.3794714	0.7687192	0.02400078	0.9742401
GE19310		-0.2342866	0.85010526	0.02402822	0.9742401
GE1125132		-0.2106642	0.8641393	0.02409106	0.9742401
GE1119981		0.4692453	1.38438508	0.0253163	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1144331		0.3905532	1.31089597	0.02540801	0.9742401
GE12467		-0.3645619	0.7767047	0.0254216	0.9742401
GE1166168	Ifi44_predicted	-0.4325765	0.74093737	0.02553126	0.9742401
GE1119215	RGD1307930_predicted	-0.2787604	0.82429897	0.02601852	0.9742401
GE1191594		-0.590998	0.6638835	0.02644257	0.9742401
GE1105443		-0.6355622	0.64368993	0.02657084	0.9742401
GE1118699		-0.26286	0.83343408	0.0266012	0.9742401
GE15516		-0.6632384	0.63145928	0.02665177	0.9742401
GE1225733		-0.4938878	0.7101089	0.02686538	0.9742401
GE1270770		-0.2272604	0.85425554	0.02735102	0.9742401
GE1101508		0.3408067	1.26646455	0.02755493	0.9742401
GE1303080		-0.2831404	0.82180021	0.02766491	0.9742401
GE19921	Sstr1	-0.2262005	0.85488336	0.02793737	0.9742401
GE14510		-0.3108878	0.80614552	0.02795853	0.9742401
GE15270		-0.232832	0.85096282	0.02799782	0.9742401
GE1137175		0.3780877	1.29961806	0.02806276	0.9742401
GE17486		-0.2664826	0.83134396	0.02808017	0.9742401
GE18102		-0.4503441	0.73186827	0.02812136	0.9742401
GE1186410		-0.1931226	0.87471043	0.0283272	0.9742401
GE14637		-0.178343	0.8837174	0.02843888	0.9742401
GE1178041		0.3114622	1.24096481	0.02854568	0.9742401
GE1251571	Edg4_predicted	-0.3689805	0.77432949	0.0287246	0.9742401
GE18232		-0.179561	0.88297164	0.02885284	0.9742401
GE12381	Clstn1_predicted	-0.1901018	0.87654387	0.02896414	0.9742401
GE13152		0.3010431	1.23203488	0.02902111	0.9742401
GE1106081		-0.5003047	0.70695745	0.02917543	0.9742401
GE18462	Cyp2u1_predicted	-0.2151565	0.86145271	0.02946445	0.9742401
GE1159255	Hspd1	0.2051425	1.15280021	0.02948937	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE18503	Me3_predicted	-0.3195752	0.80130579	0.02978319	0.9742401
GE14279		-0.5266939	0.69414362	0.02994676	0.9742401
GE13105		-0.4883532	0.71283832	0.03000325	0.9742401
GE1260146		0.427647	1.34503806	0.030679	0.9742401
GE1123692		-0.3783535	0.76931508	0.03079889	0.9742401
GE14247		-0.2478735	0.84213679	0.03081332	0.9742401
GE1246848	Itgb7	-0.4318177	0.74132717	0.03081683	0.9742401
GE12928		-0.3330872	0.79383595	0.03086871	0.9742401
GE1159779		-0.3136106	0.80462552	0.03100788	0.9742401
GE1139024		-0.2562758	0.83724642	0.03151304	0.9742401
GE15887		-0.3524338	0.78326163	0.03152537	0.9742401
GE19135	Entpd4_predicted	-0.297583	0.81361433	0.0316397	0.9742401
GE1218820		0.5701083	1.48463501	0.03170786	0.9742401
GE1104371		-0.3451147	0.78724538	0.03183656	0.9742401
GE1176403	Wwox_predicted	0.2985304	1.22989095	0.03186532	0.9742401
GE1186856		-0.2477956	0.84218226	0.03195063	0.9742401
GE1211637	C4a	0.5734645	1.4880928	0.03234322	0.9742401
GE1151346		-0.2475013	0.84235408	0.03254525	0.9742401
GE15474		0.2435477	1.18390039	0.0325953	0.9742401
GE14547		-0.2905893	0.81756804	0.03276794	0.9742401
GE1288630		-0.3873123	0.76455262	0.03290352	0.9742401
GE1246062		-0.3912451	0.76247128	0.03294445	0.9742401
GE1189819		-0.5047421	0.70478636	0.0329504	0.9742401
GE1144680		-0.2157664	0.8610886	0.03327845	0.9742401
GE1293987		-0.3511687	0.78394878	0.03330703	0.9742401
GE1259708		0.3216577	1.24976574	0.03356534	0.9742401
GE21600		0.4936291	1.4079822	0.03364345	0.9742401
GE1222235		-0.3524912	0.78323047	0.03388194	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1277074		-0.3080579	0.80772836	0.0338859	0.9742401
GE1190650		-0.5125016	0.70100586	0.03390574	0.9742401
GE1128270		-0.4793195	0.71731589	0.03414921	0.9742401
GE21912	Aif1	-0.5253236	0.69480325	0.03432657	0.9742401
GE1126064	Arl11_predicted	-0.4191834	0.7478478	0.03452025	0.9742401
GE1232143		-0.2924012	0.81654189	0.0349239	0.9742401
GE14414		-0.3472087	0.78610357	0.03537063	0.9742401
GE1247284		0.5159638	1.42994911	0.03562934	0.9742401
GE17001		0.526166	1.44009701	0.0356684	0.9742401
GE1173117		0.3654602	1.28829251	0.03632642	0.9742401
GE1192382		-0.2227293	0.85694273	0.03637531	0.9742401
GE1293979		-0.310824	0.80618117	0.0367119	0.9742401
GE1209624	Uhrf1	0.450261	1.36628741	0.03686973	0.9742401
GE20263		-0.4739208	0.72000518	0.03691329	0.9742401
GE1268278		-0.4348145	0.73978887	0.03714045	0.9742401
GE1198406	Gnat2_predicted	-0.668859	0.62900396	0.03741463	0.9742401
GE18496		-0.557783	0.67934532	0.03743741	0.9742401
GE1173886		-0.2837248	0.82146738	0.0377638	0.9742401
GE1124599		-0.3620047	0.77808264	0.03777675	0.9742401
GE13416	Slco2b1	-0.2990976	0.81276062	0.0380601	0.9742401
GE12350		-0.3055582	0.80912909	0.03832611	0.9742401
GE1288554	Kif11	0.4206398	1.33852102	0.0386304	0.9742401
GE18209		0.2619768	1.19912063	0.03867746	0.9742401
GE17021	Tk1	0.4704189	1.38551171	0.03871382	0.9742401
GE1297098	Dpp4	-0.2283824	0.85359143	0.03886528	0.9742401
GE1152954		-0.274127	0.82695057	0.03888159	0.9742401
GE1175151		-0.1917604	0.87553673	0.03890334	0.9742401
GE1258982		-0.2390345	0.84731217	0.0389722	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1110199	Cd38	0.1784692	1.13168245	0.03909561	0.9742401
GE17651		-0.3415468	0.78919472	0.03951932	0.9742401
GE14524		-0.351305	0.78387472	0.03957795	0.9742401
GE1115760		0.3689885	1.29144706	0.03974941	0.9742401
GE19223		-0.5857679	0.6662946	0.04005884	0.9742401
GE1142794		-0.283919	0.82135681	0.04007653	0.9742401
GE1299763		-0.2571726	0.83672613	0.04074291	0.9742401
GE1186313		-0.1928638	0.87486735	0.04079913	0.9742401
GE1291673		-0.2914847	0.81706078	0.04081772	0.9742401
GE200028		-0.174512	0.88606719	0.04093288	0.9742401
GE1104662	Oasl2_predicted	-0.6389367	0.64218608	0.04119843	0.9742401
GE1194766		-0.4502787	0.73190145	0.04165203	0.9742401
GE12500		-0.3045227	0.80971006	0.04185895	0.9742401
GE1175862		-0.3527432	0.78309367	0.0420169	0.9742401
GE1105472	Ifi27l	-0.3631298	0.77747608	0.04211196	0.9742401
GE1204505		-0.468106	0.72291303	0.04220638	0.9742401
GE13905		0.414719	1.33303901	0.04259022	0.9742401
GE1200043	Slc25a1	0.2896083	1.22230837	0.04269364	0.9742401
GE1192761		-0.2883668	0.81882849	0.04293735	0.9742401
GE13743		-0.1599155	0.89507749	0.04342056	0.9742401
GE1201615	Rhbg	-0.3696532	0.77396852	0.04368547	0.9742401
GE18419		-0.2620836	0.83388272	0.04378957	0.9742401
GE13612		-0.2611414	0.83442749	0.0439101	0.9742401
GE17774		-0.4088685	0.75321388	0.04411215	0.9742401
GE1213992		-0.1828536	0.88095877	0.04421127	0.9742401
GE18016		-0.1758552	0.88524261	0.04429627	0.9742401
GE20458	Psme1	-0.1833185	0.88067493	0.04449244	0.9742401
GE1207056		-0.2045272	0.86782304	0.04459425	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1179513		-0.2395164	0.84702919	0.04462256	0.9742401
GE1288320	Copeb	-0.2039315	0.86818145	0.0446442	0.9742401
GE1125563		-0.2500583	0.84086243	0.0446767	0.9742401
GE1277415		-0.2492784	0.84131712	0.04487056	0.9742401
GE14105		-0.1917472	0.87554474	0.0449693	0.9742401
GE1245685		-0.5792731	0.66930092	0.04499403	0.9742401
GE1188345		0.2455034	1.18550636	0.0450403	0.9742401
GE1182115	Lmo4_predicted	0.491357	1.40576652	0.04514024	0.9742401
GE1122708		-0.4057083	0.7548656	0.04523748	0.9742401
GE12846	Pxmp4	0.7268981	1.65507673	0.04537115	0.9742401
GE19454		0.1860222	1.13762273	0.04625462	0.9742401
GE1285906		-0.4047154	0.75538529	0.04633053	0.9742401
GE13087		-0.2580982	0.83618948	0.04635257	0.9742401
GE21525	Pold1	0.5020395	1.41621421	0.04646237	0.9742401
GE1152408		-0.4050139	0.75522902	0.04656434	0.9742401
GE13107		0.2620207	1.19915712	0.0465765	0.9742401
GE1110637		-0.2083309	0.86553802	0.04658652	0.9742401
GE20620	C1qbp	0.1852401	1.13700618	0.04660025	0.9742401
GE1164273		-0.2705607	0.8289973	0.04673149	0.9742401
GE1243886		-0.2478542	0.84214806	0.04675307	0.9742401
GE1174680		-0.2801772	0.82348987	0.04694607	0.9742401
GE1238211		-0.5739392	0.67178002	0.04707001	0.9742401
GE1222220		-0.2317228	0.85161732	0.04720861	0.9742401
GE17996		-0.3611063	0.77856732	0.04742268	0.9742401
GE1266959		-0.1711147	0.88815618	0.04768065	0.9742401
GE16098		-0.1977595	0.87190358	0.04775104	0.9742401
GE1190208		-0.2381917	0.8478073	0.04777061	0.9742401
GE13489	Capn10	0.2596568	1.19719387	0.04794066	0.9742401

A.1 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE20885	Mgat2	0.3610091	1.28432391	0.04806675	0.9742401
GE17372		0.2087064	1.1556515	0.04826464	0.9742401
GE1263701		-0.3162854	0.8031351	0.0490027	0.9742401
GE1230177		-0.4595158	0.72723029	0.04905972	0.9742401
GE15581		-0.2486042	0.84171037	0.04907714	0.9742401
GE1259471		0.3503261	1.27484876	0.0494084	0.9742401
GE17250		-0.2708068	0.82885589	0.04947148	0.9742401
GE1292531		0.5243671	1.43830247	0.04979336	0.9742401
GE1282096		-0.2171856	0.86024195	0.04980143	0.9742401
GE19716		0.4041839	1.32334012	0.04997408	0.9742401

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA)

Gene Probe	Name	logFC	FC	P.Value	Adj. P. Value
GE20556	Aqp8	0.8900115	1.853190896	0.000574123	0.9999631
GE19031	Acbd3	-0.4847266	0.714632484	0.00080867	0.9999631
GE18951	Gprc5c_predicted	-0.3872125	0.764605509	0.001763525	0.9999631
GE12548	RGD1305679_predicted	0.4213392	1.33917008	0.002188546	0.9999631
GE1212098		-0.3223663	0.799757044	0.002212695	0.9999631
GE1153287	Guca2b	-0.659428	0.63312927	0.002663984	0.9999631
GE1163784		-0.7261895	0.604498431	0.003004588	0.9999631
GE20066	Crybb1	-0.8734732	0.545831212	0.00377394	0.9999631
GE12678		0.6160921	1.532717804	0.003783799	0.9999631
GE1272052		-0.4629158	0.725518446	0.003943548	0.9999631
GE15543		0.3776416	1.299216266	0.004043971	0.9999631
GE19454		0.2943676	1.226347295	0.004274278	0.9999631
GE1241027		-0.6671691	0.629741173	0.004878517	0.9999631
GE1144374	Sdc2	-0.3711484	0.773166803	0.004930547	0.9999631
GE17322		-0.3935011	0.761279904	0.005218176	0.9999631
GE1230298		-0.6088077	0.655738406	0.005417163	0.9999631
GE14436		0.2200061	1.164738511	0.005493988	0.9999631
GE1246012		-0.6669819	0.629822892	0.00593104	0.9999631
GE1255307		-0.4978586	0.708157123	0.006584113	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P.Value**	**Adj. P. Value**
GE12956	Nmnat3_predicted	-0.270627	0.828959199	0.006584915	0.9999631
GE13636		-0.3629382	0.777579344	0.006655248	0.9999631
GE17900		-0.5425164	0.68657232	0.006801282	0.9999631
GE1162408		-0.4117131	0.751730216	0.006922321	0.9999631
GE200017		-0.291809	0.816877131	0.007186008	0.9999631
GE1195482	Ptdss1_predicted	-0.4237395	0.74548979	0.007243756	0.9999631
GE18535		-0.4046214	0.755434512	0.007285961	0.9999631
GE15306		0.3427976	1.268213465	0.007523069	0.9999631
GE13813	Lrba_predicted	-0.2692483	0.829751766	0.00779285	0.9999631
GE1231279		0.64943	1.568548349	0.008018228	0.9999631
GE1260210		-0.3845026	0.766043063	0.008466854	0.9999631
GE1262049		0.2671386	1.203418633	0.008661719	0.9999631
GE15548		0.4908447	1.405267421	0.008738199	0.9999631
GE1204676	Padi2	-0.4419606	0.736133535	0.008786402	0.9999631
GE1266336		-0.3942618	0.760878604	0.008913181	0.9999631
GE12240		-0.2729415	0.827630375	0.009274634	0.9999631
GE1203096	Tor1b_predicted	-0.3176872	0.802355111	0.010117003	0.9999631
GE17681		-0.3258648	0.797820003	0.010215621	0.9999631
GE13001		0.5638109	1.478168669	0.010744538	0.9999631
GE1105445		0.4548858	1.370674302	0.010955666	0.9999631
GE21291		0.6887518	1.611888331	0.011046448	0.9999631
GE1140170		0.6063675	1.52242114	0.011552411	0.9999631
GE1151117	Abcb1	0.9393169	1.917620052	0.011563122	0.9999631
GE13582	Arpc1a	-0.28947	0.818202585	0.011698842	0.9999631
GE1287369		0.3026099	1.233373625	0.012191823	0.9999631
GE1279714		0.2871733	1.22024708	0.01224047	0.9999631
GE1143438		0.4479374	1.364088644	0.012457784	0.9999631
GE1246654	Myo1c	-0.3029047	0.810618665	0.012679175	0.9999631
GE1217186	Shoc2_predicted	-0.4605235	0.72672251	0.012867081	0.9999631
GE1201176		0.5695681	1.484079215	0.013127253	0.9999631
GE1212897		0.3487462	1.273453429	0.013527479	0.9999631
GE200012		-0.2602411	0.834948373	0.013746157	0.9999631
GE16905	Cdc42ep5_predicted	-0.2659234	0.831666255	0.013747785	0.9999631
GE1143935	Cry1	-0.6667127	0.629940425	0.013890223	0.9999631
GE22871		0.3837182	1.304700077	0.014061753	0.9999631
GE1118248		0.4838289	1.39845022	0.01414126	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P.Value**	**Adj. P. Value**
GE20385	Adora2b	-0.3547063	0.78202883	0.014395938	0.9999631
GE13838		0.2800253	1.214216177	0.015083115	0.9999631
GE1193194		-0.5373312	0.689044374	0.015289977	0.9999631
GE14183	bsnd	-0.3658433	0.776015137	0.015360974	0.9999631
GE16132		-0.3612496	0.778489993	0.015685595	0.9999631
GE1124900	Sh2d4b_predicted	-0.3768015	0.770143131	0.016201692	0.9999631
GE1106335		0.3330791	1.259699045	0.016622762	0.9999631
GE14184	RGD1309676_predicted	-0.2900026	0.817900585	0.016831936	0.9999631
GE1258695		-0.3751718	0.771013593	0.016895229	0.9999631
GE1114268		-0.2669843	0.831054905	0.017057805	0.9999631
GE13393	Slpi	-0.4766091	0.718664785	0.017115325	0.9999631
GE22100		-0.2926268	0.816414211	0.017920395	0.9999631
GE19602		0.459422	1.374990833	0.017989761	0.9999631
GE15226		0.441595	1.358104978	0.018244546	0.9999631
GE1286367		0.5591613	1.473412411	0.018348126	0.9999631
GE1292545	Cte1	0.7873568	1.725909479	0.018408954	0.9999631
GE22871		0.3626241	1.285762429	0.019512506	0.9999631
GE13399	Kif2	-0.3282243	0.79651625	0.019533057	0.9999631
GE1269371	Ndrg2	-0.3071413	0.808241704	0.019617238	0.9999631
GE14740	Cdh3	-0.5259295	0.694511506	0.019761991	0.9999631
GE1235347		0.2433624	1.183748338	0.019794179	0.9999631
GE22872		0.2522398	1.191054806	0.020159101	0.9999631
GE1249378		0.2049008	1.152607091	0.02040691	0.9999631
GE20029	Sftpd	-0.5692444	0.673969682	0.020410108	0.9999631
GE1178454		0.2186257	1.163624599	0.02044755	0.9999631
GE1228066		-0.4782488	0.717848448	0.02062054	0.9999631
GE1138		0.3280748	1.255337076	0.020835537	0.9999631
GE1151857	Grb14	-0.4084913	0.753410842	0.020894416	0.9999631
GE1252090		0.3135208	1.242736818	0.021520717	0.9999631
GE1189819		-0.4981767	0.708000998	0.021567799	0.9999631
GE20753	Dbil5	-0.3008934	0.811749559	0.021655697	0.9999631
GE13142	St14	-0.3568416	0.780872223	0.021694076	0.9999631
GE14510		-0.3239762	0.798865095	0.021784122	0.9999631
GE13303	Slc4a7	-0.4245897	0.745050593	0.021959052	0.9999631
GE21084		0.2176693	1.162853457	0.021995805	0.9999631
GE13170		-0.4886763	0.712678694	0.022245441	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P.Value**	**Adj. P. Value**
GE1294377		0.2917357	1.224112116	0.022577121	0.9999631
GE19798	Cbs	-0.3631838	0.777446982	0.022663796	0.9999631
GE22871		0.3833943	1.304407191	0.022777521	0.9999631
GE16027		0.4235128	1.341189227	0.023039744	0.9999631
GE20211		0.4075059	1.326390795	0.023040217	0.9999631
GE15528		-0.4404133	0.736923466	0.023489058	0.9999631
GE1100961		-0.3804556	0.768194958	0.023905174	0.9999631
GE16492	RGD1311599_predicted	-0.2744031	0.826792324	0.024063022	0.9999631
GE1203640		0.3753447	1.297149443	0.024098884	0.9999631
GE16600		-0.3379321	0.791174537	0.024701289	0.9999631
GE200021		0.1957878	1.145349421	0.024709082	0.9999631
GE17535	Elovl6	0.2592295	1.196839338	0.024794622	0.9999631
GE12627		0.1950307	1.14474852	0.024869019	0.9999631
GE1129970		0.2926876	1.22492006	0.024955274	0.9999631
GE1217109		-0.4901466	0.711952749	0.025062015	0.9999631
GE1278270		0.2888808	1.221692157	0.025565041	0.9999631
GE1276666		0.2912138	1.223669369	0.0259075	0.9999631
GE16009		-0.2653994	0.831968378	0.026290604	0.9999631
GE1275884	Ndnl2_predicted	-0.3079591	0.807783678	0.026297513	0.9999631
GE1180248		0.412941	1.331397164	0.026391201	0.9999631
GE1231851		-0.3160579	0.803261759	0.026827065	0.9999631
GE21922		-0.2743161	0.826842184	0.027120316	0.9999631
GE19305		0.2691246	1.205076388	0.027323684	0.9999631
GE20102		-0.2426852	0.845170783	0.02738808	0.9999631
GE19474	Hadhb	0.2314077	1.173979894	0.02773682	0.9999631
GE1196834		0.3657755	1.288574098	0.027931137	0.9999631
GE1225974	Gpr126_predicted	-0.3179569	0.802205132	0.028050389	0.9999631
GE20876		0.2027979	1.150928252	0.028832258	0.9999631
GE1171870		-0.2884381	0.818788021	0.028901361	0.9999631
GE1141499	RGD1305326_predicted	-0.3405459	0.789742425	0.029313386	0.9999631
GE13200	Cdc42bpa	-0.378608	0.769179384	0.029990492	0.9999631
GE1184076		-0.2923949	0.816545453	0.030156334	0.9999631
GE1073413		0.3010028	1.232000465	0.030167428	0.9999631
GE16416		0.2661251	1.202573523	0.03023198	0.9999631
GE1302159		-0.3789336	0.769005808	0.030310046	0.9999631
GE1235947	RGD1311300_predicted	0.2885365	1.221400635	0.030516438	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P.Value**	**Adj. P. Value**
GE1167852	Pklr	-0.2286672	0.853422942	0.030627065	0.9999631
GE18138		0.5484632	1.46252694	0.030726577	0.9999631
GE12626	Nnt	-0.2476934	0.842241927	0.030730577	0.9999631
GE20225		0.4103768	1.329032883	0.030918465	0.9999631
GE14425	Rest	-0.2396213	0.846967608	0.030974332	0.9999631
GE1220535	RGD1305506_predicted	-0.3168396	0.802826643	0.031185	0.9999631
GE1113213		0.2723286	1.207755648	0.031200761	0.9999631
GE1181170	Vps52	-0.2642408	0.832636784	0.031366013	0.9999631
GE200001		-0.267371	0.830832179	0.031645527	0.9999631
GE15940		-0.2443677	0.844185702	0.03164665	0.9999631
GE18458		-0.3590889	0.779656798	0.031908687	0.9999631
GE19993		-0.3731845	0.772076389	0.032019688	0.9999631
GE12557	Pde4b	-0.6808806	0.623784409	0.032044568	0.9999631
GE1105115		-0.2490098	0.841473766	0.032347117	0.9999631
GE1320213	Pc	-0.393362	0.761353308	0.032429291	0.9999631
GE14741		-0.3022321	0.810996672	0.032432774	0.9999631
GE1117601		0.2938666	1.225921499	0.032734224	0.9999631
GE1148		0.2832379	1.216923012	0.032844727	0.9999631
GE1139800		-0.2628346	0.833448754	0.032965698	0.9999631
GE16776	Slc2a13	-0.3488128	0.785230001	0.03305906	0.9999631
GE20140	Scgb1a1	-0.6056835	0.657159966	0.033099432	0.9999631
GE21842	Sdc2	-0.2722017	0.828054885	0.033409204	0.9999631
GE1280431		-0.3076595	0.807951445	0.033471792	0.9999631
GE1250625		-0.5279085	0.693559471	0.033785097	0.9999631
GE14549		-0.3799813	0.768447551	0.033846955	0.9999631
GE1151413		0.2788745	1.213248015	0.033876247	0.9999631
GE19865	S100a4	0.375556	1.29733944	0.033893214	0.9999631
GE1157514		-0.2944773	0.815367693	0.034323345	0.9999631
GE19342		-0.1778612	0.884012577	0.034381	0.9999631
GE15917		0.3577205	1.281399649	0.034418275	0.9999631
GE1073421		0.380476	1.301771288	0.034566246	0.9999631
GE1154004		0.3629882	1.286086964	0.034631071	0.9999631
GE20144		0.4316287	1.348755367	0.034984792	0.9999631
GE18548		0.1967988	1.146152331	0.035267902	0.9999631
GE14862		0.212328	1.158556177	0.035761905	0.9999631
GE1247664		-0.3166692	0.802921472	0.035772287	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P.Value**	**Adj. P. Value**
GE1262726		-0.2688541	0.829978517	0.035901544	0.9999631
GE1264588		0.3747913	1.296651968	0.035925748	0.9999631
GE20373	Cfl1	-0.2090834	0.86508668	0.036063689	0.9999631
GE1127390		0.2494725	1.188772379	0.036381063	0.9999631
GE19200	Cad	-0.2020142	0.869336003	0.036487599	0.9999631
GE1144267		0.2603044	1.197731392	0.036697054	0.9999631
GE1138		0.30039	1.23147727	0.036760894	0.9999631
GE18304		0.2152317	1.160890341	0.036846115	0.9999631
GE1186652	Egln2	-0.2178549	0.859842961	0.036910066	0.9999631
GE1186410		0.2059737	1.153464577	0.036910643	0.9999631
GE12658	RGD1311909_predicted	-0.2765312	0.825573633	0.036959116	0.9999631
GE12578		0.315671	1.24459038	0.037171721	0.9999631
GE1073419		0.32275	1.250712329	0.037271941	0.9999631
GE1246003		-0.3082078	0.807644439	0.037471336	0.9999631
GE1123520		-0.2756993	0.82604982	0.037960038	0.9999631
GE1150575		-0.29914	0.81273673	0.038052953	0.9999631
GE200025		-0.2465481	0.842910816	0.038068087	0.9999631
GE200014		-0.1911521	0.875905965	0.038122204	0.9999631
GE1203511	Ccl28	-0.4550046	0.729507846	0.038209754	0.9999631
GE1204323	Knsl7	-0.4224814	0.746140177	0.038248892	0.9999631
GE1176901		-0.3883781	0.763988009	0.038252426	0.9999631
GE21454		-0.26657	0.831293594	0.038255604	0.9999631
GE18022		0.2404593	1.181368705	0.03831027	0.9999631
GE1291225	Stim1_predicted	-0.3858947	0.765304241	0.038484917	0.9999631
GE20705		0.7139505	1.640289542	0.038511895	0.9999631
GE1142		0.4045269	1.323654776	0.03866336	0.9999631
GE14220		-0.2493412	0.841280494	0.038945283	0.9999631
GE21437		-0.337328	0.791505895	0.038988414	0.9999631
GE1281798		-0.3589681	0.779722083	0.039277741	0.9999631
GE15175	Ercc3_predicted	-0.2061726	0.866833852	0.039388195	0.9999631
GE19144	Elmo2_predicted	-0.2715673	0.828419087	0.039465975	0.9999631
GE1134805	Cln5_predicted	-0.2410991	0.846100475	0.03964733	0.9999631
GE21591		0.2043749	1.152187012	0.039828902	0.9999631
GE1130949		0.2630018	1.19997288	0.039869491	0.9999631
GE200009		-0.2284284	0.853564216	0.040066261	0.9999631
GE21099		0.2635022	1.200389163	0.040253524	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P.Value	Adj. P. Value
GE1170956		0.273677	1.208884992	0.040465627	0.9999631
GE15095		0.2665174	1.202900573	0.040771514	0.9999631
GE13535		0.2075608	1.154734195	0.040775482	0.9999631
GE17565	Thrap3_predicted	-0.2229219	0.856828338	0.041686311	0.9999631
GE1265780		0.6851805	1.607903139	0.041845279	0.9999631
GE1214098	Cacna1d	-0.2718372	0.828264121	0.041901825	0.9999631
GE1122496		0.4268328	1.344279191	0.042000253	0.9999631
GE18659		-0.2346367	0.849898994	0.042151833	0.9999631
GE15233		0.2182458	1.163318226	0.042180704	0.9999631
GE200023		-0.2491863	0.841370826	0.042282667	0.9999631
GE15102	Ralgps2_predicted	-0.2539463	0.838599398	0.042843519	0.9999631
GE1240685	Rab10	-0.2324883	0.85116557	0.042880084	0.9999631
GE18945		0.2560845	1.194233132	0.043028934	0.9999631
GE17796	Srrm1_predicted	-0.2894425	0.818218181	0.043334235	0.9999631
GE1138771		0.2303497	1.173119271	0.043608506	0.9999631
GE15275		0.1895077	1.140374512	0.043727481	0.9999631
GE17454		-0.2149528	0.861574347	0.043903181	0.9999631
GE1142		0.3698128	1.292185149	0.044141132	0.9999631
GE1110163		-0.2994083	0.812585598	0.044164681	0.9999631
GE1320213	Pc	-0.390466	0.762883148	0.044434295	0.9999631
GE1201171		0.2562139	1.194340252	0.044448087	0.9999631
GE1209747		-0.263471	0.833081185	0.044456034	0.9999631
GE1149749		-0.6133817	0.653662709	0.044681656	0.9999631
GE1221142		-0.3427462	0.788538882	0.044962279	0.9999631
GE15856		0.3653822	1.288222861	0.045288096	0.9999631
GE21698	Ralb	-0.1748191	0.885878593	0.045397568	0.9999631
GE1160340		0.2412508	1.182017012	0.045456291	0.9999631
GE18912		-0.3090162	0.807192011	0.045795627	0.9999631
GE1170451		0.3777396	1.299304523	0.045919946	0.9999631
GE1265640		-0.2832975	0.821710724	0.045957019	0.9999631
GE1231692		-0.2655968	0.83185455	0.04602813	0.9999631
GE1258950		-0.4609054	0.726530163	0.046481084	0.9999631
GE1217295		0.2075568	1.154730993	0.046728789	0.9999631
GE1073419		0.3127416	1.242065796	0.046991701	0.9999631
GE22105	Tp53	-0.2933525	0.816003644	0.047016327	0.9999631
GE12477	Usp7_predicted	-0.2449354	0.843853581	0.047089685	0.9999631

A.2 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (mRNA) (continued)

Gene Probe	Name	logFC	FC	P.Value	Adj. P. Value
GE21192	Adam6	-0.3138761	0.804477457	0.047458279	0.9999631
GE1111582	Tfb1m	-0.3544901	0.782146033	0.047672542	0.9999631
GE1248341	RGD1305506_predicted	-0.2562264	0.837275085	0.047679035	0.9999631
GE21708		-0.2744232	0.826780805	0.047697622	0.9999631
GE1173651		0.2616682	1.19886416	0.047796564	0.9999631
GE15799	Fmo2	-0.4444338	0.734872668	0.047798489	0.9999631
GE18533		0.3347714	1.261177555	0.048154511	0.9999631
GE14328		0.2048867	1.152595826	0.048262325	0.9999631
GE1143046		0.318032	1.246628845	0.048323773	0.9999631
GE13768		-0.2153279	0.861350367	0.048398624	0.9999631
GE1291117		-0.3259658	0.797764151	0.048906151	0.9999631
GE13709		-0.2715503	0.828428849	0.049126685	0.9999631
GE1240530	Trim32_predicted	-0.2563232	0.837218908	0.049306091	0.9999631
GE1223994		0.3416971	1.26724643	0.049418625	0.9999631
GE1147		0.2712119	1.206821162	0.049876152	0.9999631
GE1179198		0.1695252	1.124688282	0.049982988	0.9999631

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1276405	Ifi47	0.680025	1.602167518	6.88E-12	8.14E-08
GE1284808		1.47563047	2.781051517	6.27E-10	2.13E-06
GE16473	Ifi1_predicted	0.7321331	1.66109329	6.38E-10	2.13E-06
GE18264		0.95059859	1.932674379	7.21E-10	2.13E-06
GE14232	Cxcl9	1.36889815	2.582732361	2.78E-09	0.0000066
GE18258	Irf7_predicted	1.13157275	2.190974586	3.76E-09	0.0000074
GE1104662	Oasl2_predicted	1.37670246	2.596741601	4.56E-09	0.0000077
GE21219		0.96308833	1.949478616	0.000000010	0.0000155
GE1204505		0.94520629	1.925464187	0.000000012	0.0000162
GE1152228		0.57541652	1.490107609	0.000000097	0.0001122
GE1246848	Itgb7	0.79810487	1.738815511	0.000000104	0.0001122
GE1167182	RT1-S3	0.67188076	1.593148512	0.000000155	0.0001530
GE22080		0.88474826	1.846442402	0.000000601	0.0005468
GE19916		0.51613255	1.430116375	0.000001024	0.0008655
GE13281		0.86462604	1.82086762	0.000001335	0.0010527

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1157676		0.58881791	1.50401391	0.000001721	0.0011891
GE1111927		0.96456953	1.951481154	0.000001786	0.0011891
GE1105472	Ifi27l	0.83097217	1.778883672	0.000001810	0.0011891
GE1286280		0.43773037	1.354471812	0.000002573	0.0016019
GE19041		0.61490251	1.531454506	0.000010528	0.0062257
GE1304439		-0.41325663	0.750926375	0.000011899	0.0067017
GE1100195		0.37125637	1.293478765	0.000015662	0.0084196
GE1268278		0.6423366	1.560855086	0.000040501	0.0208266
GE1111552		0.68416284	1.606769343	0.000049218	0.0242541
GE20152	Cd74	0.74037641	1.670611657	0.000059484	0.0281408
GE1118248		-0.59794197	0.660695778	0.000155153	0.0705766
GE1166168		0.51669612	1.43067514	0.000202180	0.0885624
GE1244655		0.84870179	1.800879673	0.000279572	0.1180892
GE14234		0.69499465	1.61887843	0.000298923	0.1219090
GE13149		0.42204632	1.339826619	0.000326534	0.1254575
GE1148695		0.47676794	1.391622529	0.000328839	0.1254575
GE20140		0.68731822	1.610287422	0.000371344	0.1313852
GE1273147		0.45327168	1.369141618	0.000376953	0.1313852
GE13923		0.43709163	1.353872265	0.000386355	0.1313852
GE20458		0.20241565	1.150623348	0.000388812	0.1313852
GE1224306		0.69150572	1.614968158	0.000402562	0.1322529
GE13393		0.53758654	1.451542225	0.000488595	0.1561786
GE20012		0.42305374	1.340762534	0.000524762	0.1633252
GE1304692		0.75191977	1.684032254	0.000548503	0.1663371
GE1172984		0.37738313	1.298983522	0.000585060	0.1729877
GE1299763		0.29870163	1.230036929	0.000635383	0.1832847
GE1160237		0.75401643	1.686481427	0.000776109	0.2169724
GE1178209		0.4271416	1.344566956	0.000788857	0.2169724
GE1214832		-0.63531608	0.643799749	0.000855175	0.2298670
GE1215793		0.30968122	1.239433803	0.001042509	0.2739946
GE22138		0.59060353	1.505876577	0.001069144	0.2748862
GE14567		0.65592299	1.575623664	0.001227379	0.3088557
GE15448		0.23002609	1.172856159	0.001284625	0.3165263
GE1125357		0.90072608	1.867005374	0.001381364	0.3334161
GE15443		0.37964648	1.301023012	0.001455676	0.3443256
GE1114268		0.25936269	1.196949836	0.001573613	0.3649239

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1105693		0.52409949	1.438035698	0.001625004	0.3695948
GE1266336		0.38741885	1.308051053	0.001674185	0.3735960
GE1237531		0.57586909	1.490575126	0.001873553	0.4103428
GE1200712		0.34831895	1.273076355	0.001930994	0.4152339
GE21027		0.57578352	1.490486719	0.002039813	0.4300833
GE1271436		0.54560884	1.459636204	0.002072778	0.4300833
GE1286312		0.29058692	1.223137776	0.002551968	0.5203814
GE1288491		0.26926831	1.205196435	0.002635908	0.5283879
GE18535		0.34612654	1.271143177	0.002811686	0.5542302
GE19415		0.23974165	1.180781195	0.003000667	0.5817850
GE21912		0.53864001	1.452602542	0.003063552	0.5843972
GE1210278		0.57123915	1.485799195	0.003258792	0.6117736
GE12887		0.37898203	1.300423949	0.003370088	0.6227817
GE15169		0.40768694	1.326557251	0.003864168	0.7031002
GE1249754		0.31936967	1.247785258	0.003993698	0.7156585
GE1134279		0.24491012	1.185018942	0.004128962	0.7240839
GE18664		0.17848883	1.131697852	0.004217982	0.7240839
GE20655		0.30536241	1.235729018	0.004224384	0.7240839
GE1158013		0.19993291	1.148644938	0.004435281	0.7493723
GE1172237		0.28147695	1.215438545	0.004558021	0.7592636
GE12477		0.20997034	1.156664404	0.004764180	0.7825828
GE1282096		0.20528545	1.152914439	0.005000460	0.8101430
GE1168606		0.26345677	1.200351364	0.005095011	0.8143067
GE20892		0.27619796	1.21099924	0.005618364	0.8855577
GE1163784		0.44932124	1.365397713	0.005690571	0.8855577
GE16043		0.27719243	1.211834287	0.005866074	0.8920870
GE1159850		0.49536201	1.409674435	0.005883384	0.8920870
GE1266727		-0.56255659	0.677101214	0.006152192	0.9133835
GE19110		0.29186179	1.224219107	0.006247079	0.9133835
GE19154		0.19760697	1.146794563	0.006277777	0.9133835
GE18055		0.47048657	1.385576695	0.006336547	0.9133835
GE18061		0.23366359	1.175817039	0.006413073	0.9133835
GE16111		0.25001046	1.189215737	0.006509580	0.9133835
GE1159508		0.42853723	1.345868289	0.006670118	0.9133835
GE17308		0.35022686	1.274761064	0.006676923	0.9133835
GE14490		0.22049166	1.165130587	0.006718894	0.9133835

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE17900		0.35262333	1.276880338	0.006866981	0.9228805
GE1103820		0.29687052	1.228476721	0.006951611	0.9228805
GE13790		0.22521943	1.16895503	0.007022850	0.9228805
GE17525		-0.13776705	0.908924867	0.007112110	0.9243399
GE16343		0.41169852	1.330251029	0.007285306	0.9365577
GE18690		0.25645621	1.194540866	0.007472377	0.9502774
GE1134955		-0.26904561	0.829868349	0.007641467	0.9580205
GE1191279		0.28252514	1.216321943	0.007695269	0.9580205
GE21698		0.13939372	1.101442147	0.007799473	0.9604432
GE15812		0.249697	1.18895738	0.007877145	0.9604432
GE1215909		0.29373692	1.225811309	0.008393572	0.9992956
GE12871		0.17516644	1.129094658	0.008462129	0.9992956
GE17797		0.41323236	1.331666074	0.008673314	0.9992956
GE20621		0.58123723	1.496131755	0.008748670	0.9992956
GE1146173		0.23776518	1.17916465	0.008880302	0.9992956
GE1243274		0.3186169	1.247134358	0.008932855	0.9992956
GE15748		0.17373104	1.127971831	0.009030580	0.9992956
GE13154		0.3346635	1.261083234	0.009072214	0.9992956
GE1282521		-0.46233212	0.725812033	0.009239729	0.9992956
GE1118758		-0.50701285	0.70367792	0.009715708	0.9992956
GE13724		0.37186725	1.294026579	0.009744973	0.9992956
GE1276531		0.21970607	1.164496312	0.010330180	0.9992956
GE1178454		0.21318301	1.159242996	0.010524400	0.9992956
GE1244087		0.23601962	1.177738806	0.010556890	0.9992956
GE1233836		-0.30822355	0.807635622	0.010594740	0.9992956
GE1276045		0.40062828	1.320082669	0.010759810	0.9992956
GE14639		-0.22670433	0.854584864	0.011150330	0.9992956
GE1142287		0.25504649	1.193374198	0.011220170	0.9992956
GE13795		0.32736251	1.254717441	0.011475800	0.9992956
GE1252674		0.28748255	1.220508676	0.011972650	0.9992956
GE1119199		0.20934308	1.156161615	0.012128860	0.9992956
GE1130008		0.2909865	1.223476592	0.012719010	0.9992956
GE1277062		0.22042387	1.16507584	0.012782780	0.9992956
GE1197697		-0.19780159	0.871878138	0.012910530	0.9992956
GE1188737		0.22788014	1.17111288	0.012916680	0.9992956
GE1204323		0.30169771	1.232594031	0.013039590	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE12877		0.14184342	1.103313988	0.013048270	0.9992956
GE1275377		-0.23216532	0.851356144	0.013149630	0.9992956
GE1134441		-0.4131461	0.750983908	0.013320820	0.9992956
GE1269110		-0.32528885	0.798138571	0.013517210	0.9992956
GE13271		-0.70471705	0.613562812	0.013610370	0.9992956
GE1102169		0.21219219	1.15844712	0.013698840	0.9992956
GE1241640		0.23251512	1.174881392	0.013717900	0.9992956
GE15231		0.24520905	1.185264506	0.013769440	0.9992956
GE1245278		-0.30746242	0.808061823	0.013940510	0.9992956
GE1260210		0.23618916	1.177877218	0.013944310	0.9992956
GE1171466		-0.46234226	0.725806932	0.014086220	0.9992956
GE13744		0.26804094	1.204171552	0.014296320	0.9992956
GE1191917		0.20919341	1.156041677	0.014392610	0.9992956
GE20917		0.36163332	1.284879727	0.014468900	0.9992956
GE15305		0.23688939	1.178449054	0.014731860	0.9992956
GE1267470		-0.19477036	0.873711957	0.014904230	0.9992956
GE200009		-0.168971	0.889476873	0.015041800	0.9992956
GE1298223		0.19166552	1.142081432	0.015050930	0.9992956
GE1177391		0.19279483	1.142975778	0.015233060	0.9992956
GE1193689		0.25442152	1.192857345	0.015261520	0.9992956
GE12653		0.15856854	1.116179102	0.015267550	0.9992956
GE1280376		0.45686589	1.372556835	0.015494860	0.9992956
GE1200454		0.2207077	1.165305075	0.015497250	0.9992956
GE20373		0.15657121	1.114634884	0.016015020	0.9992956
GE1133737		0.6905776	1.613929545	0.016193000	0.9992956
GE20844		0.38788373	1.308472614	0.016238040	0.9992956
GE1209969		0.21048135	1.157074173	0.016386040	0.9992956
GE1100172		-0.60325547	0.658266886	0.016390180	0.9992956
GE21916		0.26202742	1.199162705	0.016421670	0.9992956
GE1169834		0.3381844	1.264164669	0.016517200	0.9992956
GE1264588		-0.37521688	0.770989501	0.017328400	0.9992956
GE1265734		-0.27544904	0.826193125	0.017464760	0.9992956
GE1305560		0.33332078	1.259910087	0.017483980	0.9992956
GE1306802		0.3056516	1.235976746	0.017528990	0.9992956
GE200018		0.29259153	1.224838494	0.017699440	0.9992956
GE1287874		0.13484456	1.097974509	0.017704750	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE13212		0.16202359	1.118855396	0.017789880	0.9992956
GE15046		0.15126016	1.110539078	0.018173900	0.9992956
GE1142745		0.25085956	1.189915858	0.018647680	0.9992956
GE1191468		0.1844334	1.136370591	0.018894430	0.9992956
GE14909		0.36536424	1.288206825	0.018938320	0.9992956
GE1106921		-0.18263308	0.881093434	0.019035490	0.9992956
GE1244623		-0.5267758	0.694104217	0.019205500	0.9992956
GE13399		0.20707676	1.154346834	0.019233730	0.9992956
GE1211329		0.19140551	1.141875618	0.019308500	0.9992956
GE1161593		0.35434538	1.278405376	0.019678190	0.9992956
GE1122228		0.24080186	1.181649248	0.019682100	0.9992956
GE1225484		0.15113052	1.11043929	0.019814090	0.9992956
GE18503		0.25611828	1.194261095	0.020226490	0.9992956
GE1103809		-0.49034028	0.711857176	0.020692040	0.9992956
GE1255307		0.36269706	1.285827454	0.020716060	0.9992956
GE20452		0.16140187	1.118373336	0.020916060	0.9992956
GE1228817		0.25394699	1.192465056	0.020992840	0.9992956
GE1243124		-0.2588026	0.835781309	0.021081160	0.9992956
GE1117573		-0.50233249	0.705964484	0.021094750	0.9992956
GE15263		0.23171731	1.174231863	0.021124410	0.9992956
GE21679		0.43207203	1.349169893	0.021714250	0.9992956
GE17448		0.30866988	1.238565255	0.021716390	0.9992956
GE15366		0.14506857	1.105783209	0.021778570	0.9992956
GE13702		0.17989124	1.132798484	0.021918010	0.9992956
GE18548		-0.13674897	0.909566503	0.021934810	0.9992956
GE1201425		-0.2478733	0.842136908	0.022274720	0.9992956
GE1295381		0.26857753	1.20461951	0.022332920	0.9992956
GE200029		0.14868267	1.108556783	0.022393610	0.9992956
GE18558		0.26354196	1.200422246	0.022564060	0.9992956
GE18376		0.12953277	1.093939362	0.023180540	0.9992956
GE21743		0.17953688	1.132520276	0.023337570	0.9992956
GE17851		-0.28740593	0.819374029	0.023412730	0.9992956
GE14105		-0.15075233	0.900780605	0.023425320	0.9992956
GE19685		0.15948878	1.116891297	0.023526090	0.9992956
GE17147		0.21487631	1.160604405	0.023677750	0.9992956
GE14928		0.30073376	1.231770737	0.023852570	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE17990		0.21550733	1.161112153	0.023889460	0.9992956
GE1128249		0.53502156	1.448963808	0.024417070	0.9992956
GE15337		0.13759464	1.100069475	0.024433330	0.9992956
GE1276722		-0.2135867	0.862390565	0.025104180	0.9992956
GE1301162		0.20650703	1.153891065	0.025269780	0.9992956
GE18081		0.17274238	1.127199112	0.025286270	0.9992956
GE14774		0.26585239	1.202346224	0.025499210	0.9992956
GE15176		0.14918089	1.108939678	0.025564780	0.9992956
GE12771		0.16067754	1.117811978	0.025620700	0.9992956
GE12637		0.21073222	1.157275394	0.025683750	0.9992956
GE1104692		0.25301964	1.191698798	0.025794830	0.9992956
GE1177763		-0.583486	0.667349304	0.025986060	0.9992956
GE1149749		0.54711267	1.461158486	0.026581090	0.9992956
GE12384		0.24321544	1.183627762	0.026647240	0.9992956
GE18539		0.17735299	1.130807212	0.026823800	0.9992956
GE1107302		0.24403117	1.184297199	0.026853490	0.9992956
GE1152784		-0.25192958	0.839772482	0.026973620	0.9992956
GE1240571		-0.22507636	0.855549741	0.027041290	0.9992956
GE1154563		0.33908785	1.264956567	0.027280720	0.9992956
GE1121497		-0.26756169	0.83072237	0.027388960	0.9992956
GE1126625		0.18055935	1.133323203	0.027494110	0.9992956
GE13677		-0.28481558	0.820846531	0.027542960	0.9992956
GE1129281		-0.21732881	0.860156567	0.027547870	0.9992956
GE12940		0.31355285	1.242764426	0.027629750	0.9992956
GE200009		-0.13010551	0.913764621	0.027848330	0.9992956
GE14319		0.21402529	1.159919987	0.028006810	0.9992956
GE1144374		0.19740477	1.146633846	0.028034500	0.9992956
GE18486		0.29754861	1.229054261	0.028068350	0.9992956
GE18350		0.13092687	1.094996964	0.028123980	0.9992956
GE17619		0.1634907	1.119993764	0.028287370	0.9992956
GE1281387		0.26518814	1.201792764	0.028498710	0.9992956
GE14375		-0.22853945	0.853498516	0.028692260	0.9992956
GE1243675		0.24476725	1.184901595	0.028877450	0.9992956
GE13414		0.2421085	1.182719945	0.028979630	0.9992956
GE14425		0.1562828	1.114412079	0.029394610	0.9992956
GE1172464		0.18384581	1.135907857	0.029578660	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1237829		-0.23665001	0.84871377	0.030131510	0.9992956
GE1301839		-0.400497	0.75759725	0.030158750	0.9992956
GE1151884		0.20442695	1.152228581	0.030308870	0.9992956
GE18813		0.22069921	1.165298218	0.030583430	0.9992956
GE13418		0.14171045	1.103212302	0.030644240	0.9992956
GE1174275		-0.23369969	0.850451171	0.030728930	0.9992956
GE13733		0.24440624	1.184605131	0.030887330	0.9992956
GE1181587		-0.47836741	0.717789433	0.031206350	0.9992956
GE200015		0.15809747	1.115814705	0.031635220	0.9992956
GE13907		0.24500601	1.185097708	0.031986750	0.9992956
GE12485		0.21351314	1.159508294	0.032038390	0.9992956
GE19111		0.22635202	1.16987308	0.032053630	0.9992956
GE1126416		0.65896713	1.578951799	0.032088750	0.9992956
GE1291356		0.2129278	1.159037946	0.032697970	0.9992956
GE1237093		-0.24182274	0.845676187	0.032776530	0.9992956
GE1185422		0.21950225	1.164331806	0.032992070	0.9992956
GE1272179		0.20885142	1.15576767	0.033153860	0.9992956
GE14122		0.20967227	1.156425455	0.033333020	0.9992956
GE1261494		0.12476813	1.09033248	0.033525720	0.9992956
GE1245049		0.16471078	1.120941338	0.033585360	0.9992956
GE17842		-0.14632661	0.90354815	0.033773850	0.9992956
GE13699		0.22420827	1.168136017	0.033896630	0.9992956
GE18144		0.30143423	1.232368943	0.033938880	0.9992956
GE13172		0.22425243	1.168171773	0.034004030	0.9992956
GE1174142		-0.19233464	0.875188302	0.034021420	0.9992956
GE1100961		0.27945957	1.213740135	0.034650890	0.9992956
GE200028		0.14680596	1.107115669	0.034797440	0.9992956
GE1271024		-0.23460096	0.849920049	0.034936490	0.9992956
GE1256552		0.30555142	1.235890923	0.034996060	0.9992956
GE1128514		0.16297381	1.119592564	0.035274040	0.9992956
GE1197604		0.20802023	1.155101982	0.035582710	0.9992956
GE17578		0.22125112	1.165744094	0.035654850	0.9992956
GE1178943		0.14415735	1.105085006	0.035702280	0.9992956
GE18087		0.3947864	1.314748089	0.035738200	0.9992956
GE1267743		0.24881748	1.188232768	0.036174420	0.9992956
GE1115541		0.24290439	1.183372595	0.036477670	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1187970		0.14379001	1.104803665	0.036573360	0.9992956
GE1213767		0.19391706	1.143865211	0.036615920	0.9992956
GE200022		-0.13539075	0.910423215	0.036619730	0.9992956
GE1101442		-0.391613	0.762276867	0.036629300	0.9992956
GE1180166		0.31044084	1.240086572	0.036656030	0.9992956
GE12484		0.20709655	1.154362669	0.036857080	0.9992956
GE1298524		0.26965206	1.205517054	0.037007310	0.9992956
GE18390		0.22024205	1.164929018	0.037009710	0.9992956
GE200006		-0.11184318	0.925405013	0.037189130	0.9992956
GE1190650		0.37806074	1.299593776	0.037259440	0.9992956
GE1127390		-0.16119309	0.894285203	0.037488540	0.9992956
GE1235269		0.18754214	1.138821898	0.037595750	0.9992956
GE1128939		-0.16369673	0.892734614	0.037709310	0.9992956
GE1279845		-0.27515874	0.826359389	0.037769640	0.9992956
GE1227016		0.13847463	1.100740681	0.037914310	0.9992956
GE21120		0.13440635	1.097641056	0.037933520	0.9992956
GE1232179		0.16056168	1.117722213	0.038012040	0.9992956
GE1117543		0.277329	1.211949008	0.038030240	0.9992956
GE1195241		-0.22377158	0.856323854	0.038037890	0.9992956
GE15906		0.18305718	1.135287097	0.038125740	0.9992956
GE1277415		-0.1725329	0.887283534	0.038908470	0.9992956
GE19417		0.16057369	1.117731517	0.039264820	0.9992956
GE1199065		0.15988499	1.117198073	0.039294460	0.9992956
GE1163435		0.30953175	1.239305398	0.039420020	0.9992956
GE14529		-0.13821431	0.908643129	0.039427130	0.9992956
GE1073414		-0.17733179	0.884337033	0.039866920	0.9992956
GE1163169		-0.53904544	0.688226124	0.040164190	0.9992956
GE17311		0.15718211	1.115106969	0.040212280	0.9992956
GE19031		0.24844098	1.187922716	0.040247680	0.9992956
GE1182666		0.15737918	1.115259301	0.040480730	0.9992956
GE20602		0.35661355	1.280416834	0.040924850	0.9992956
GE1188754		-0.27004676	0.829292667	0.041277570	0.9992956
GE15789		0.1925815	1.14280678	0.041594920	0.9992956
GE1259119		0.1765912	1.130210267	0.041619680	0.9992956
GE1126881		0.33977503	1.265559231	0.041648220	0.9992956
GE19277		-0.19041332	0.876354617	0.041674200	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	Name	logFC	FC	P. Value	Adj. P. Value
GE1241596		0.14949733	1.109182938	0.041729600	0.9992956
GE1183240		0.15428586	1.112870606	0.041750560	0.9992956
GE13982		-0.16894469	0.889493094	0.041849610	0.9992956
GE1278937		0.1683107	1.123741887	0.041936310	0.9992956
GE1272962		-0.33765882	0.791324418	0.042067970	0.9992956
GE1255350		-0.35120834	0.783927239	0.042128350	0.9992956
GE1144667		0.27884232	1.213220953	0.042153380	0.9992956
GE1291064		-0.20578118	0.867069066	0.042245720	0.9992956
GE18800		0.20918996	1.156038912	0.042370410	0.9992956
GE1136896		-0.31121319	0.805963724	0.042480890	0.9992956
GE1141455		0.18131955	1.133920543	0.042826420	0.9992956
GE1293934		-0.22774106	0.853970974	0.043234150	0.9992956
GE1295453		0.24968256	1.188945479	0.043465660	0.9992956
GE1220885		0.35923988	1.282749871	0.043486890	0.9992956
GE13104		0.43209651	1.349192786	0.043815720	0.9992956
GE1218115		0.28556752	1.21888965	0.043885340	0.9992956
GE13080		0.2259943	1.169583043	0.044102940	0.9992956
GE1281798		0.2171861	1.16246405	0.044805500	0.9992956
GE12904		0.21974063	1.164524208	0.044827090	0.9992956
GE16720		0.19571969	1.14529535	0.044895540	0.9992956
GE1162408		0.32327023	1.251163413	0.044938680	0.9992956
GE1297897		-0.15190315	0.90006235	0.044993140	0.9992956
GE15097		0.24528551	1.185327325	0.045271670	0.9992956
GE12315		0.25534279	1.193619318	0.045403350	0.9992956
GE18168		0.16214367	1.118948525	0.045403710	0.9992956
GE200014		0.12537826	1.09079369	0.045737090	0.9992956
GE1303900		0.25808526	1.195890469	0.046045240	0.9992956
GE15955		0.1836898	1.135785029	0.046224750	0.9992956
GE1273349		0.23018688	1.172986883	0.046225340	0.9992956
GE1276969		-0.29950283	0.812532357	0.046431650	0.9992956
GE17887		0.12673011	1.091816277	0.046837800	0.9992956
GE1293370		0.21753059	1.162741658	0.047000300	0.9992956
GE12978		0.16888241	1.124187291	0.047055930	0.9992956
GE1237324		0.30312055	1.233810261	0.047629540	0.9992956
GE1169082		0.15928381	1.116732626	0.047659260	0.9992956
GE1273269		0.261812	1.198983662	0.047933500	0.9992956

A.3 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (mRNA) (continued)

Gene Probe	**Name**	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
GE1215837		0.16525861	1.12136707	0.047934020	0.9992956
GE1199619		0.22632789	1.169853514	0.048014650	0.9992956
GE1241345		-0.35705618	0.780756089	0.048280260	0.9992956
GE1298834		0.23920828	1.180344736	0.048556490	0.9992956
GE1165606		-0.62237847	0.649599096	0.048598050	0.9992956
GE1147202		0.18022248	1.133058602	0.048701240	0.9992956
GE200006		0.10190338	1.073188411	0.048863220	0.9992956
GE13646		-0.16234585	0.893570925	0.049170800	0.9992956
GE15499		-0.192605	0.875024308	0.049264340	0.9992956
GE1073408		-0.18903113	0.877194621	0.049604430	0.9992956
GE1257574		0.20431253	1.152137202	0.049724470	0.9992956
GE200014		0.10959333	1.078924064	0.049922980	0.9992956

A.4 Differentially Expressed miRNA for All AOM Groups with Contrast Between Omega 3 and Omega 6 (Part 1)

miR-532-3p	**miR-532-5p**	**miR-146-5p**	**miR-18-5p**	**miR-199-3p**
ZNF514	MRPL18	IGSF1	NEDD9	KLHL3
DDOST	RASSF5	KBTBD4	TMEM170B	SERPINE2
SLC10A5	GBP1	PSMA4	FBXL3	TMSB4X
GSG1L	SMIM10	CDKN2AIP	RAB9A	PAWR
LBH	DENND6A	ZBTB2	RORA	NLK
P2RX6	CRIPT	IRAK1	HEATR5A	QKI
CYTH1	KRAS	SLC10A3	ERI1	VAMP3
HMGA2	NDP	HNRNPD	INADL	GNA12
NEDD8	ZKSCAN1	ZDHHC13	FAM3C	PAK4
CARHSP1	ZNF250	NOVA1	KCNA1	DEPDC1B
CBX1	CXCL1	TRAF6	MAP7D1	BCAR3
KIAA0087	CLVS2	TDRKH	DIRAS2	NOVA1
RAD51	TTC14	AC012215.1	BBX	CDK7
CSF3	CCDC64	TMEM120B	ESR1	PON2
TCF23	CXCL2	RARB	F3	FAM199X
PABPN1	CYCS	LFNG	TTPAL	CYB5R4
RAB9A	ZMPSTE24	CD80	TMEM2	CD2AP
ETNK2	ERO1L	ACKR2	HMGCS1	CISD2
SUMO1	CCNG1	NUMB	ZNF367	MCFD2
MDM4	DDHD1	KLF7	CREBL2	UPRT

A.5 Differentially Expressed miRNA for All AOM Groups with Contrast Between Omega 3 and Omega 6 (Part 2)

miR-199-5p	**miR-17-5p/20-5p/93-5p/106-5p/519-3p**	**miR-19-3p**	**miR-646**	**miR-451**
ZNF776	CTDSPL	CTDSPL2	PTCD3	OSR1
ZNF439	OPCML	KBTBD8	DGCR2	ATF2
ZNF544	RBM24	ZMYND11	CMC4	MIF
ZNF791	MARCH4	CHIC1	CYB561D1	PSMB8
ZNF788	PDCD1LG2	C11orf96	CDK8	TSC1
ZNF627	GPR6	QKI	ARL2	S1PR2
ZIK1	GPR137C	PMEPA1	TUT1	C11orf30
ZNF772	HN1	LONRF1	MTRNR2L6	AEBP2
ZNF709	NAGK	ZBTB18	TFPI2	GK
ZNF584	ZBTB7A	RNF11	PNP	VAPA
ZNF625	ENPP5	HBP1	AP5S1	DKFZP779J2370
ZNF256	HAUS8	KCNA4	CDK5RAP1	CDKN2D
ZNF547	RAB22A	NEUROD1	STK33	TBX1
MAP3K11	BTG3	SPTSSB	ZNF880	PRICKLE2
LIN7C	VSX1	DTNA	LCE6A	SAMD4B
PVRL2	PKD2	MPPED2	RP1-241P17.4	MEF2D
ZNF561	CCL1	ATL2	FKBP1B	YWHAZ
DDR1	IRF9	DNAJA2	RP3-422G23.4	PMM2
RP11-1396O13.13	CYBRD1	TAOK1	CAAP1	BTBD9
ZNF23	BRMS1L	PLA2G10	VAMP2	FBXO33

A.6 Differentially Expressed miRNA for All Saline Groups with Contrast Between Omega 3 and Omega 6 (Part 1)

miR-126-3p.1	**miR-126-3p.2**	**miR-191-5p**	**miR-214-5p**
PLXNB2	S1PR2	WSB1	ANKRD65
RGS3	PCNXL4	TMC7	GPR173
ADAM9	KLF10	TMOD2	GPR39
HERPUD1	ANKRD44	PLCD1	KCNC4
KANK2	AKT2	TAF5	TFDP1
SLC7A5	MXD1	ZBTB34	PGS1
FBXO33	ADAMTS9	CALN1	TMEM51
PLK2	CD97	NEURL4	HDC
SPRED1	RASA2	SATB1	PARS2
EGFL7	KANK2	EGR1	WASL

A.6 Differentially Expressed miRNA for All Saline Groups with Contrast Between Omega 3 and Omega 6 (Part 1) (continued)

miR-126-3p.1	**miR-126-3p.2**	**miR-191-5p**	**miR-214-5p**
CRK	TLN2	MAPRE3	RP11-422N16.3
CAMSAP1	RER1	HOXB2	E2F2
IRS1	PIK3R2	CDK6	SLC43A1
BAK1	LGALSL	OXSR1	OXER1
PEX5	LRRC1	TCF7L2	C8orf46
DIP2C	SMURF2	SLC16A2	SOX4
SDC2	KCNJ2	SOX4	C14orf1
ITGA6	PDGFRB	BDNF	BUB3
EFHD2	PIK3R1	EMX2	HMG20A
GNA13	PLAGL2	BRMS1L	RBM24

A.7 Differentially Expressed miRNA for All Saline Groups with Contrast Between Omega 3 and Omega 6 (Part 2)

miR-34-5p/449-5p	**miR-10-5p**	**miR-103-3p/107**	**miR-375**
MDM4	BDNF	RNF38	C3orf38
HCN3	ARSJ	PPP6R2	ELAVL4
FAM76A	CRLF3	TRIAP1	SLC16A2
SCN2B	TFAP2C	MED26	GREM2
SYT1	HOXA3	NPAS3	EIF4G3
FAM167A	VWC2L	ANO3	RPN1
RRAS	SLC6A19	ARIH2	ZFP36L2
VAMP2	RNF186	NEK10	LRAT
FUT9	SOBP	ARMC1	POU3F1
MYCN	TFRC	AGFG1	MXI1
SLC23A3	SMAP1	TFRC	HNF1B
ANK3	KPNA5	CACNA2D1	HOXA5
SAMD12	GATA6	GPRIN3	ISL2
FOSL1	HCN1	SPATS2L	FOXF1
FKBP1B	FIGN	TPD52	YBX1
DLL1	DAZAP1	WDFY4	PLEKHA3
PVRL1	HOXB3	TMEM47	LRP5
FLOT2	NR6A1	AGO4	UBE2E2
CDIP1	KLHL29	GPC6	DESI1
E2F5	NCOR2	ZHX1	IFT20

A.8 Differentially Expressed miRNA for All Groups with Contrast Between AOM and Saline

miR-146-5p	**miR-34-5p/449-5p**	**miR-378-3p**
IGSF1	MDM4	PDIA4
KBTBD4	HCN3	IMPG1
PSMA4	FAM76A	TMED7-TICAM2
CDKN2AIP	SCN2B	SDAD1
ZBTB2	SYT1	GOLT1A
IRAK1	FAM167A	FAM179B
SLC10A3	RRAS	PAG1
HNRNPD	VAMP2	RSPH4A
ZDHHC13	FUT9	KIAA1429
NOVA1	MYCN	CD226
TRAF6	SLC23A3	BMP2
TDRKH	ANK3	HNRNPA1
AC012215.1	SAMD12	TSPAN17
TMEM120B	FOSL1	SBDS
RARB	FKBP1B	IL6ST
LFNG	DLL1	EIF4G3
CD80	PVRL1	ARL10
ACKR2	FLOT2	MED19
NUMB	CDIP1	METTL4
KLF7	E2F5	MROH8

A.9 Differentially Expressed Genes for All AOM Groups with Contrast Between Omega 3 and Omega 6 (miRNA)

Detector	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
hsa-miR-532-4380928	0.00701853	1.00487673	0.00233185	0.3119179
hsa-miR-146b-4373178	-0.2297951	0.85275601	0.00354452	0.3119179
hsa-miR-18a-4373118	0.05749058	1.04065408	0.01396658	0.7531421
hsa-miR-199b-4373100	0.01834607	1.01279772	0.01711687	0.7531421
hsa-miR-93-4373012	0.73182825	1.66074233	0.02386978	0.8402164
hsa-miR-19b-4373098	3.13076372	8.75898512	0.03996073	0.9166653
hsa-miR-646-4381002	0.04120474	1.02897272	0.04148122	0.9166653
hsa-miR-451-4373209	0.00842481	1.00585671	0.04696899	0.9166653

A.10 Differentially Expressed Genes for All Saline Groups with Contrast Between Omega 3 and Omega 6 (miRNA)

Detector	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
hsa-miR-126-4378064	0.51364256	1.42765022	0.0077908	0.9572851
hsa-miR-191-4373109	-1.7805971	0.29106291	0.02507017	0.9572851
hsa-miR-214-4373085	0.03794389	1.02664962	0.03129959	0.9572851
hsa-miR-449-4373207	-0.0223759	0.98460987	0.03417153	0.9572851
hsa-miR-10b-4373152	-0.0259776	0.98215485	0.04424008	0.9572851
hsa-miR-107-4373154	-0.0341676	0.97659509	0.04522291	0.9572851
hsa-miR-375-4373027	-1.8956274	0.2687567	0.04707649	0.9572851

A.11 Differentially Expressed Genes for All Groups with Contrast Between AOM and Saline (miRNA)

Detector	**logFC**	**FC**	**P. Value**	**Adj. P. Value**
hsa-miR-146b-4373178	-0.1439161	0.905059121	0.00267136	0.4701594
hsa-miR-34a-4373278	-0.0075334	0.994791829	0.03016017	0.9692872
hsa-miR-378-4373024	0.12615585	1.091381769	0.04899143	0.9692872