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Stage-specific signaling pathways during murine testis development and spermatogenesis: a pathway-based analysis to quantify developmental dynamics

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Abstract

Shifting the field of developmental toxicology towards evaluation of pathway perturbation requires a quantitative definition of normal developmental dynamics. This project examined a publicly available dataset to quantify pathway dynamics during testicular development and spermatogenesis and anchor toxicant-perturbed pathways within the context of normal development. Genes significantly changed throughout testis development in mice were clustered by their direction of change using *K*-means clustering. Gene Ontology terms enriched among each cluster were identified using MAPPfinder. Temporal pathway dynamics of enriched terms were quantified based on average expression intensity for all genes associated with a given term. This analysis captured processes that drive development, including the peak in steroidogenesis known to occur around gestational day 16.5 and the increase in meiosis and spermatogenesis-related pathways during the first wave of spermatogenesis. Our analysis quantifies dynamics of pathways vulnerable to toxicants and provides a framework for quantifying perturbation of these pathways.

Keywords

testicular development; spermatogenesis; quantitative pathway analysis

Introduction

Increasing rates of reproductive disorders that have origins in early reproductive development demonstrate the need for methods to characterize and quantify perturbations of developmental processes in gonadal development and spermatogenesis. For example, there is mounting evidence for a consistent decline in semen quality in recent decades, accompanied by an increasing prevalence of male reproductive disorders, including hypospadias, undescended testes, and testicular cancer [1–6]. All of these adverse reproductive health outcomes are manifestations of testicular dysgenesis syndrome (TDS), a

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set of conditions believed to have common origins during early gonadal development [7]. Early testicular development and spermatogenesis are very sensitive processes that depend on a series of precisely timed steps regulated by hormonal cues and germ cell microenvironments [8, 9]. These processes in male reproductive development are therefore particularly vulnerable to perturbation by genetic and environmental factors [10, 11]. Indeed, the recent increase in TDS related conditions has been hypothesized to be a result of environmental factors that can influence early male reproductive development, such as exposure to endocrine disrupting chemicals [7].

Exploration of the complex interaction of environmental and genetic factors underlying reproductive disorders requires a systems-based framework for characterizing normal and perturbed pathway dynamics during critical windows of male reproductive development. The field of toxicology is increasingly shifting towards characterization of pathway perturbation as a sensitive indicator of toxicity [12]. A quantitative framework for measuring shifts from normal pathway dynamics would facilitate quantification of pathway perturbation by toxicants. Furthermore, incorporation of *in vitro* models into chemical screening, underscores the need to anchor pathway dynamics captured in these *in vitro* models to pathway dynamics driving *in vivo* development. The first step in being able to place pathway perturbation measured *in vivo* and *in vitro* within the context of normal development is to define normal pathway dynamics *in vivo* in an easily translatable, quantitative framework.

Fortunately, much of the data needed to provide this baseline characterization of normal developmental dynamics is available in publicly available datasets. Microarray-based high-throughput gene expression analysis has proven to be an effective method for studying the changes in gene expression associated with the growth and development of mammalian tissues [13, 14]. Many of the genetic drivers of male reproductive development have been characterized through mouse knockout models [15, 16] and global gene expression analysis [17–21]. The Griswold lab at Washington State University has employed microarray-based gene expression analysis to characterize dynamic changes in global gene expression patterns over the course of several particularly sensitive processes of male reproductive development in mice [17, 18]. The group identified genes with changing expression patterns throughout gonadal differentiation and development and the first wave of spermatogenesis [17, 18]. The gene expression profiles observed by Small *et al* reiterated the known functional activities of each cell type, and suggested the involvement of novel genes in the maturation of the testis and differentiation of germ cells. Their temporal microarray study provides a valuable resource for evaluating biological factors that influence testis maturation and spermatogenesis. However, as with all microarray data, the functional interpretation of such a vast set of genomic data presents a major challenge. In order to elucidate the biological consequences of these expression changes in single genes, gene expression data must be integrated with quantitative information on functional changes in whole gene networks and developmental signaling pathways over time.

Gene ontology (GO) analysis is a powerful tool for translating a vast amount of genomic data into a description of functional changes in gene networks and signaling pathways. The GO approach has been successfully combined with pathway analysis to generate an

unbiased determination of the statistical significance of changes observed in pathways of interest [22–25]. For example, previous GO analysis of testicular gene expression has successfully identified pathways that are significantly changed throughout murine spermatogenesis [26]. However, standard GO analysis results in a list of enriched pathways with no quantitative description of how these pathways are changed. In addition these approaches did not retain quantitative information on the expression of individual genes and are limited to the evaluation of only two experimental dimensions.

In order to address the need to quantify changes in pathway dynamics through time or in response to an environmental exposure, our lab developed the GO-Quant approach [27]. GO-Quant incorporates gene expression data with Gene Ontology analysis in MAPPfinder [22] to calculate the average intensity of expression of all significantly altered genes associated with a given GO term. This allows the quantitative evaluation of the dynamics of entire gene pathways along a third dimension, such as developmental stage or toxicant dose. We first applied this quantitative pathway-based approach in a published dose- and time-dependent genomic dataset [28] and found that our systematic approach quantitatively described the degree to which functional gene systems changed across dose or time course [27, 29]. We have subsequently used our quantitative pathway analysis for a genome-wide assessment of phthalate toxicity in an *in vitro* rat testis co-culture model [30] and for an assessment of time- and dose-dependent methylmercury toxicity in developing mouse embryos undergoing neurulation [31].

In the current study, we applied our established quantitative pathway-based approach to a publicly available dataset of murine male reproductive development [18] to quantify the dynamic functional changes in biological processes that characterize normal testicular development and the first wave of spermatogenesis *in vivo*. Through this analysis we demonstrate that our approach can quantitatively illustrate pathway dynamics throughout a complex developmental process *in vivo*, successfully capturing well characterized developmental milestones. The result provides a framework for quantifying perturbation of normal developmental pathways *in vivo* as well as anchoring emerging *in vitro* models of male reproductive development to *in vivo* pathway dynamics.

Materials and Methods

Gene Expression Data Set

For this analysis we obtained publically available temporal mouse genomic data during early testis development (gestational days (GD) 11.5, 12.5, 14.5, 16.5, and 18.5) and the first wave of spermatogenesis (postnatal days (PND) 0, 3, 6, 8, 10, 14, 18, 20, 30, 35, and 56). Gene expression intensity in testicular tissue at each timepoint was quantified using Affymetrix MGU74Av2, Bv2, and Cv2 arrays. Detailed methods of sample collection and microarray processing are available in the original papers [17, 18]. NCBI's gene expression omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) was used to retrieve the raw dataset.

Identification and Clustering of Significantly Changed Genes

Microarray analysis was conducted based on our established quantitative pathway-based approach as shown in Figure 1 [27]. Significantly changed probes were identified using BRB ArrayTools, developed by Dr. Richard Simon and the BRB ArrayTools Development Team. Data were normalized by gcRMA normalization and log2 transformed. In order to identify significantly changed probes, we conducted an ANOVA class comparison across timepoints. Genes that were significantly altered ($p < 0.001$) across time were selected and *K*-means cluster analysis was used to group genes based on the similarity of their patterns of mean expression through time. Since there are generally two directions of gene expression changes at a certain time (either up-regulation or down-regulation within a specific gene category), the average of these two different directions of gene expression alteration would mask the degree of absolute change in a pathway. For pathway analysis, we therefore separated significantly changed genes into two groups with patterns of expression tending towards consistent up- or down-regulation across time based on *K*-means cluster analysis [32].

Identification of Enriched GO terms

We applied MAPPfinder to identify enriched Gene Ontology (GO) terms at $p < 0.001$ [33] in up- and down-regulated probes at each timepoint. Enriched GO terms were ranked by *Z*-score and permutation *p*-value [33]. As previously described, the *Z*-score, a statistical measure of significance for gene expression in a given group, was calculated by subtracting the number of genes expected to be randomly changed in a GO term from the observed number of changed genes in that GO term. This value was then divided by the standard deviation of the observed number of genes under a hypergeometric distribution. The equation is written out as

$$Zscore = (r - n \cdot (R/N)) / ((n \cdot (R/N)) (1 - (R/N)) (1 - ((n-1)/(N-1)))^{1/2} \quad (1)$$

where N is the total number of genes measured, R is the total number of genes meeting the criterion that the gene be significantly changed based on an *F* test at significant $p \leq 0.001$ value, n is the total number of genes in each specific GO term, and r is the number of genes meeting the criterion in this specific GO term. Complete lists of GO terms enriched among significantly up- or down-regulated genes are available as supplemental data (Supplemental Tables 1 and 2).

Quantification of Pathway Dynamics Throughout Development

To quantify the dynamic changes in GO terms through time, we used GO-Quant to link enriched GO terms to the expression values of all the significantly up- or down-regulated genes contained within the pathway. By incorporating gene expression data into the pathway analysis, we could compute the average intensity of expression among genes in each enriched GO term at each timepoint. These values at individual timepoints were then normalized to 'average' expression across time by subtracting the average expression intensity of genes in the GO term across all the timepoints. This yielded a log2 ratio of mean expression at each timepoint relative to the average mean expression across all timepoints. Plotting the log2 ratio for each GO term through time illustrates temporal pathway dynamics

throughout development. GO terms included in the figures and tables presented here are the ten biological processes with the highest Z-scores that are relevant to each of the dominant categories selected.

Results

Our quantitative pathway analysis (summarized in Figure 1A) translates a vast amount of information on expression of single genes into a quantitative summary of temporal changes in activity across entire gene networks and biological processes described by GO terms (Figure 1B). Of the approximately 36,000 probes on the array, 9,599 probes were significantly changed through time. Significant genes were clustered into two groups based on their general expression trends using K-means clustering. This resulted in segregation of significantly changed genes into two sets: one set with overall upward trends in expression and another set with overall downward trends in expression. We then used MAPPFinder to identify GO terms describing biological processes that were enriched among each of these two gene clusters. We found 308 GO terms enriched among genes with downwards trends in expression and 112 GO terms enriched among genes with upward trends in expression. For each of these enriched GO terms, GO-Quant facilitated generation of a quantitative temporal summary of average gene expression dynamics at each timepoint among genes significantly changed in the pathway through time.

GO Terms Significantly Enriched Among Genes with Upward Trends in Expression

The GO terms most significantly over represented among genes with increased expression are dominated by terms associated with spermatogenesis and meiosis (Table 1). Indeed, 12 of the top 20 (60%) of significantly enriched GO terms ranked by Z-score are directly relevant to spermatogenesis or meiosis (Supplemental Table 1). Quantitative pathway analysis reveals that average gene expression in GO terms related to meiosis (Figure 2B) begins increasing around PND 3, slightly preceding the spermatogenesis and spermatid maturation signal. We see a dramatic increase in the average expression of genes in GO terms relating to spermatogenesis and spermatid development throughout testis development, capturing gene expression dynamics that are closely aligned in time with known developmental outcomes (Figure 2A). Average expression of genes involved in a host of GO terms relating to spermatid development and differentiation begin a gradual increase on PND 10 with the emergence of spermatocytes, increase around PND 20 when spermatids appear, and remain high through PND 56 as the first wave of spermatogenesis is completed and spermatozoa are produced. Simultaneously, average expression in GO terms associated with a panel of pathways related to ubiquitin mediated processes (Figure 2C) and epigenetic processes (Figure 2D) increases in parallel, with over a quarter of the genes associated with chromatin remodeling and ubiquitin cycle changing significantly through spermatogenesis.

GO Terms Significantly Enriched Among Genes with Downward Trends in Expression

The dominant themes in GO terms enriched among genes with decreasing expression through time include developmental processes, metabolic processes, developmental signaling pathways, mitosis, and steroid regulation (Table 2). Quantitative pathway analysis

illustrates dynamic changes in GO terms related to steroid regulation throughout testis development. For example, average gene expression in sterol metabolic and biosynthetic processes peak at GD 16.5, decrease before birth, and decrease further after spermatid development commences (Figure 3A). Several other catabolic and metabolic pathways, including lipid metabolism, follow a similar pattern of expression (Figure 3B).

Our analysis also reveals dynamic changes of signal transduction pathways throughout spermatogenesis (Figure 3C). Average gene expression of many developmental signaling pathways, including BMP, Notch, and MAPK signaling, are elevated towards the end of gestation and during the earliest stages of spermatogenesis, peaking between GD 16.5 and PND 3. Average expression in these developmental signaling pathways decreases dramatically between PND 14 and PND 18, as testicular formation and development give way to functional adult tissue.

GO terms for developmental processes (Figure 3D) and mitosis (Figure 3E) display a similar pattern of dramatic upregulation during peak testicular development, dropping off as testes mature and spermatogenesis progresses. For example, pathways involved in cell growth and differentiation, mitosis, and cellular morphogenesis and development, are generally elevated during gestation and the first week of life. Then, around PND 8, expression of genes in these pathways begins a gradual decrease. This decrease corresponds to the end stages of testis development and maturation and the beginning of spermatogenesis.

Discussion

Our systematic pathway-based approach distills complex genomic data into a quantitative description of pathway dynamics over the course of gonadal development and spermatogenesis. This analysis successfully captures well characterized developmental processes, offering a quantitative framework for assessing normal temporal dynamics of reproductive development and measuring any perturbation of these dynamics that could lead to pathology. Finally, by defining *in vivo* pathway dynamics, this analysis facilitates evaluation of the ability of emerging *in vitro* systems to capture important developmental pathways.

Pathway analysis captures dynamics of key events known to drive testicular development and spermatogenesis

The trends highlighted by our analysis are consistent with well characterized developmental processes described in the literature. For example, the notable peak in expression of pathways related to steroidogenesis and hormonal regulation observed in our analysis at GD 16.5 corresponds to the well characterized peak in testosterone known to drive male reproductive development [16, 34]. Furthermore, in this dataset, specific genes that are widely recognized in the literature for their roles in steroidogenesis, including, star, 3 β -HSD, and C/EBP β [35, 36] follow temporal expression patterns that are consistent with the average expression patterns reflected in pathway analysis. The significant increase in steroidogenesis during this phase has previously been identified as a critical initiator of testis development and masculinization of the fetus [16, 34]. Expression of a host of genes in the mouse reproductive tract has been shown to be modulated by estrogen and testosterone [37].

The dramatic proliferation of somatic cells that occurs early in testis development plays a role in promoting the male fate by increasing the number of SRY-producing cells and marks a key difference between male and female development [38]. Accordingly, in this analysis, we see that pathways that promote proliferation and mitosis are expressed in developing testes and then downregulated as functionally mature testes begin spermatogenesis. Conversely, meiosis in the testis does not begin until around PND5, with the initiation of the first wave of spermatogenesis [39]. This is clearly illustrated at the pathway level in our analysis. In addition, several meiosis-specific genes described in the literature, such as the family of synaptonemal complex proteins [40] follow expression trends consistent with overall pathway trends.

The onset of meiosis in this analysis is followed by a dramatic increase in expression of a host of specific genes known to play an important role in spermatogenesis. For example, by PND 30 there is a sharp increase in gene expression of protamines 1 and 2 and the transition proteins that facilitate the switch between histones and sperm-specific protamines [40]. General expression dynamics of GO terms related to spermatogenesis are consistent with expression dynamics for these genes with well-characterized roles in spermatogenesis.

During spermatogenesis, chromatin restructuring facilitates sperm compaction and regulation of DNA methylation [41, 42]. Precise regulation of these epigenetic processes is essential for sperm development as well as for appropriate expression of the paternal genome in the developing embryo [42]. The ubiquitin system plays an important role in performing the histone modifications that underlie this chromatin restructuring [43]. The prominent increase in expression of genes associated with ubiquitin mediated processes and epigenetic processes that is observed concurrently with spermatogenesis in our analysis illustrates the temporal dynamics of these well-described regulatory processes.

Our analysis also successfully captures the important role of signal transduction pathways in regulating development. At least 17 highly conserved signaling pathways are now recognized for their central role in guiding developmental processes [12, 44]. Many of these signaling pathways are specifically implicated in guiding the processes of gonadal differentiation and testicular development [45] and germ cell differentiation [46]. In the current analysis, we capture the increased expression of genes involved in these signal transduction pathways throughout gestation and testicular maturation. These signaling pathways are then downregulated as testis tissue achieves maturation and begins the process of meiosis and spermatogenesis.

Pathway analysis can facilitate discovery of new roles for pathways dynamically changed in testicular development

In addition to accurately illustrating these well characterized developmental processes, quantitative pathway analysis is also a powerful tool for providing new insight into the stagespecific regulation of signaling pathways involved in the unique and complex processes of gonadal differentiation and spermatogenesis. The precise balance of these signaling pathways is hypothesized to play a key role in male development by initiating the differentiation of the fetal Leydig cells that produce masculinizing hormones [47]. The current analysis shows a wide range of signal transduction pathways that go through

dramatic changes in expression in testicular tissue over the course of the first wave of spermatogenesis. Further investigation of the fluctuations in these signaling pathways over time could provide insight into their roles in the regulation and maintenance of spermatogenesis.

We also find that pathways related to cellular metabolism of lipids and proteins follow the same pattern of expression as steroidogenesis pathways during spermatogenesis. If these pathways are in fact linked, this is consistent with the hypothesis that cellular metabolism pathways are a target of androgen action, allowing androgens to modulate the testis environment to promote spermatogenesis [16]. These observations illustrate that our pathway-based approach can provide quantitative information on the dynamic changes seen in a range of signaling pathways over the course of development. Deeper exploration of this pathway analysis may reveal additional novel pathways that are important in reproductive development.

Benefits and drawbacks of the quantitative pathway-based approach

There are many benefits to applying a pathway based approach to analyze temporal gene expression data. For example, we reduce the potential for overlooking key pathways whose subtle changes have large effects by considering the pathway as a whole as opposed to individual genes. We also reduce the amount of statistical noise, as entire gene networks are far less likely than individual genes to be significantly increased simply by chance. It is important to note that while average pathway expression provides an informative summary of pathway dynamics driving developmental processes, this method is not ideal for identification and characterization of single genes that serve as key developmental regulators. In addition, the quality of a pathway-based analysis is limited by the quality of the curation of each pathway. Indeed, there are several pathways enriched in our analysis (e.g. heart development and neural tube development) that are likely to be an artifact of genes that have simultaneous roles in multiple pathways or signal transduction that is highly conserved across a diverse range of developmental processes.

Applications for developmental toxicology: defining sensitive phases of development and generating complex, context dependent Adverse Outcome Pathways

In addition to providing insight into normal gonadal development and spermatogenesis, this quantitative gene ontology analysis offers a new lens through which to evaluate developmental pathology. This will be a particularly valuable tool for our field of developmental toxicology. Understanding the dynamics of signaling pathways can further define key phases of susceptibility to environmental factors. For example quantitative characterization of the pathway dynamics that drive steroid regulation can shed light on key windows of susceptibility of this process to endocrine disrupting chemicals [7, 48].

The challenge of regulatory chemical testing requirements and the vast number of chemicals yet to be tested will require innovative computational toxicology methods [49, 50]. Toxicity-related alterations in gene transcription and biological pathway dynamics have been proposed as valuable metrics for chemical risk assessment that could be generated by highthroughput methods [51, 52]. To that end, a quantitative pathway-based approach can be

applied to predict and measure the effects of chemical exposure on signaling pathway dynamics. This approach is a powerful way to quantify changes in the developmental dynamics of signaling pathways in response to genetic mutations and environmental factors. Changes in the peaks, slopes and duration of the dynamic expression patterns of signaling pathways in response to toxicant exposure will provide a sensitive measure of reproductive developmental toxicity and offer insight into mechanisms of toxicity. Quantitative pathway-based characterization of signaling pathway dynamics could also inform mathematical models for *in silico* simulation of the specific impacts of gene changes or environmental exposures on developmental outcomes. Furthermore, quantifying the perturbation of developmental pathway dynamics in response to toxicant exposures could facilitate articulation of “Adverse Outcome Pathways”, which are emerging as an increasingly valuable tool for translating toxicological data for risk assessment [53]. Therefore, in addition to shedding light on normal developmental dynamics, this quantitative pathway-based approach provides a framework for quantifying deviation from normal developmental processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Quantitative pathway analysis of gene expression through time in testis development concisely summarizes pathway dynamics across development
- The result of this analysis successfully captures and quantifies key processes of male reproductive development described in the literature
- This approach provides a framework for quantifying perturbation of normal developmental dynamics by environmental factors

A.

B.

Figure 1. Microarray Analysis Pipeline for Quantification of Pathway Dynamics

A) Summary of analysis pipeline B) Illustration of data analysis process. Starting with single gene expression in testis through time (Shima et al, 2004) we normalized data and identified significantly changed genes using BRB Arraytools software, clustered genes with significantly increasing or decreasing expression through time using Multiple Experiment Viewer software (MEV), then used GO Quant software to identify GO terms enriched among these clusters and average the expression of all significantly changed genes in that pathway to produce a quantitative summary of gene expression dynamics in each GO term through time.

Figure 2. Quantified Pathway Dynamics of GO Terms that Significantly Increase Through Time
 GO terms enriched among genes with significantly increasing expression ($p < 0.001$) through time were identified through MAPPfinder. Significantly enriched GO terms ($p < 0.001$) were ranked by Z-score and dominant themes among these terms were identified as A) Spermatogenesis, B) Meiosis, C) Ubiquitin Mediated Processes, and D) Epigenetic Processes. Dynamic change in GO terms related to each dominant theme are plotted here as the ratio of average Log2 intensity of expression of all significantly changed genes at each timepoint in a GO term over Average Log2 intensity across all timepoints for that GO term. Corresponding stages of spermatogenesis, including spermatogonia (SPG), spermatocyte (SPC), spermatid (SPT), and spermatozoa (SPZ) are indicated along the X axis.

Figure 3. Quantified Pathway Dynamics of GO terms that Significantly Decrease Through Time
 GO terms enriched among genes with significantly decreasing expression ($p < 0.001$) through time were identified through MAPPfinder. Significantly enriched GO terms ($p < 0.001$) were ranked by Z-score and dominant themes among these terms were identified as A) Steroid Regulation B) Cellular Metabolism C) Developmental Signaling Pathways, D) Developmental Processes, and E) Meiosis. Dynamic change in GO terms related to each dominant theme are plotted here as the ratio of average Log2 intensity of expression of all significantly changed genes at each timepoint in a GO term over Average Log2 intensity across all timepoints for that GO term. Corresponding stages of spermatogenesis, including spermatogonia (SPG), spermatocyte (SPC), spermatid (SPT), and spermatozoa (SPZ) are indicated along the X axis.

Table 2.1
GO Terms Enriched Among Genes With Significantly Increased Expression Through Time

GOID	GO Name	# Genes in GO	% of Present Genes Changed	% of Genes Present on Array	Z-Score	Parametric p-value
Spermatogenesis and Sperm Maturation						
48232	male gamete generation	194	53.3	70.6	11.935	4.0E-05
7283	spermatogenesis	194	53.3	70.6	11.935	4.0E-05
7276	gamete generation	278	41.8	66.2	9.601	5.1E-05
30317	sperm motility	15	75	106.7	6.427	3.7E-05
48515	spermatid differentiation	36	56.2	88.9	6.202	7.7E-05
7286	spermatid development	34	56.7	88.2	6.067	3.3E-05
1539	ciliary or flagellar motility	6	100	66.7	4.574	1.7E-06
48240	sperm capacitation	5	100	60	3.961	3.3E-05
Meiotic Cell Cycle						
7127	meiosis I	32	63.2	59.4	5.597	1.9E-04
51327	M phase of meiotic cell cycle	76	37.7	69.7	4.309	1.5E-04
7126	meiosis	76	37.7	69.7	4.309	1.5E-04
51321	meiotic cell cycle	77	37	70.1	4.21	1.5E-04
7131	meiotic recombination	17	75	23.5	3.212	6.4E-06
7128	meiotic prophase I	4	60	125	2.677	1.0E-07
7130	synaptonemal complex assembly	6	60	83.3	2.677	1.1E-04
Ubiquitin Mediated Processes						
6512	ubiquitin cycle	433	25.3	83.1	4.837	7.7E-05
6511	ubiquitin-dependent protein catabolic process	165	28.9	69.1	3.767	6.9E-05
19941	modification-dependent protein catabolic process	167	28.4	69.5	3.654	6.9E-05
43632	modification-dependent macromolecule catabolic process	167	28.4	69.5	3.654	6.9E-05
6464	protein modification process	1609	19.5	78.3	3.535	8.7E-05
Epigenetic Regulation						
43687	post-translational protein modification	1375	19.1	79.1	2.895	8.5E-05

GOID	GO Name	# Genes in GO	% of Present Genes Changed	% of Genes Present on Array	Z-Score	Parametric p-value
6338	chromatin remodeling	52	28.9	73.1	2.169	8.6E-05
31497	chromatin assembly	142	25.4	44.4	2.026	3.6E-05
6333	chromatin assembly or disassembly	180	23.6	49.4	1.945	7.0E-05

Table 2.2
GO Terms Enriched Among Genes With Significantly Decreased Expression Through Time

GOID	GO Name	# Genes in GO	% of Present Genes Changed	% of Genes Present on Array	Z-Score	Parametric p-value
Steroid Regulation						
16125	sterol metabolic process	73	44.1	80.8	2.997	7.3E-05
6694	steroid biosynthetic process	75	46.3	72	3.237	5.7E-05
6695	cholesterol biosynthetic process	25	55	80	2.846	4.1E-05
8203	cholesterol metabolic process	67	43.4	79.1	2.729	6.0E-05
16126	sterol biosynthetic process	31	50	83.9	2.67	7.4E-05
8202	steroid metabolic process	145	36.5	79.3	2.358	9.8E-05
Cellular Metabolism						
8112	metabolic process	8113	28.8	76.1	4.835	1.3E-04
44238	primary metabolic process	7338	29	75.1	4.83	1.3E-04
43170	macromolecule metabolic process	6411	28.8	74.7	3.91	1.3E-04
44237	cellular metabolic process	7311	28.9	75.4	4.651	1.3E-04
9059	macromolecule biosynthetic process	899	34.8	65.5	4.476	1.5E-04
19538	protein metabolic process	3404	29.4	74	3.263	1.3E-04
44249	cellular biosynthetic process	646	33.1	74.9	3.156	1.4E-04
44255	cellular lipid metabolic process	542	33.2	81.2	3.063	1.4E-04
19320	hexose catabolic process	91	44.2	57.1	2.839	1.3E-04
46365	monosaccharide catabolic process	91	44.2	57.1	2.839	1.3E-04
Developmental Processes						
32502	developmental process	3292	31.8	75.6	6.268	1.2E-04
32989	cellular structure morphogenesis	485	39.4	77.9	5.61	7.9E-05
902	cell morphogenesis	485	39.4	77.9	5.61	7.9E-05
9887	organ morphogenesis	457	39.2	82.1	5.49	8.6E-05
9790	embryonic development	362	39.2	94.5	5.229	6.1E-05
35295	tube development	128	46.9	100	5.146	5.3E-05

GOID	GO Name	# Genes in GO	% of Present Genes Changed	% of Genes Present on Array	Z-Score	Parametric p-value
48731	system development	1704	32.5	74.9	4.824	1.0E-04
35239	tube morphogenesis	83	48.9	106	4.683	6.2E-05
48513	organ development	1345	32.2	76.4	4.077	1.0E-04
50793	regulation of developmental process	256	38.8	85.5	4.039	7.9E-05
Developmental Signaling Pathways						
8593	regulation of Notch signaling pathway	5	100	80	3.304	2.0E-05
48013	ephrin receptor signaling pathway	3	100	133.3	3.304	4.2E-05
30509	BMP signaling pathway	26	57.1	80.8	3.139	1.5E-04
7266	Rho protein signal transduction	102	41.5	80.4	3.002	1.6E-04
43405	regulation of MAPK activity	77	44.4	70.1	2.929	9.7E-05
35023	regulation of Rho protein signal transduction	72	42.2	88.9	2.781	1.8E-04
7265	Ras protein signal transduction	172	37.4	71.5	2.66	1.7E-04
9966	regulation of signal transduction	411	32.1	78.8	2.171	1.6E-04
187	activation of MAPK activity	48	43.8	66.7	2.164	9.9E-05
7219	Notch signaling pathway	51	40.9	86.3	2.113	5.6E-05
Mitotic Cell Cycle						
51301	cell division	218	42.9	90.8	5.155	1.7E-04
278	mitotic cell cycle	249	39.6	81.1	4.133	1.3E-04
7067	mitosis	168	41.7	85.7	4.043	1.4E-04
87	M phase of mitotic cell cycle	170	41.5	86.5	4.039	1.4E-04
8283	cell proliferation	648	35.3	63	3.925	9.8E-05
22402	cell cycle process	646	34.9	69.2	3.923	1.4E-04
7049	cell cycle	762	33.7	74.4	3.773	1.3E-04
6275	regulation of DNA replication	19	70	52.6	3.083	1.5E-04
51726	regulation of cell cycle	461	33.5	60.3	2.523	1.3E-04
74	regulation of progression through cell cycle	459	33.5	59.9	2.509	1.3E-04

Table 2.3
Full list of GO Terms Enriched Among Genes With Significantly Increased Expression Through Time

GOID	GO Name	# Genes in GO	%Genes Changed	%Genes Present	Z-Score	Parametric p-value
48232	male gamete generation	194	53.3	70.6	11.94	4.0E-05
7283	Spermatogenesis	194	53.3	70.6	11.94	4.0E-05
19953	sexual reproduction	327	45	67.9	11.87	4.6E-05
3	Reproduction	492	35.5	65.2	9.62	5.2E-05
7276	gamete generation	278	41.8	66.2	9.60	5.1E-05
9566	Fertilization	48	62.9	72.9	7.55	2.0E-05
7338	single fertilization	48	61.8	70.8	7.27	2.1E-05
30317	sperm motility	15	75	106.7	6.43	3.7E-05
48515	spermatid differentiation	36	56.2	88.9	6.20	7.7E-05
7286	spermatid development	34	56.7	88.2	6.07	3.3E-05
7127	meiosis I	32	63.2	59.4	5.60	1.9E-04
6457	protein folding	238	29.5	72.7	4.84	1.2E-04
6512	ubiquitin cycle	433	25.3	83.1	4.84	7.7E-05
35036	sperm-egg recognition	12	66.7	100	4.78	1.5E-07
1539	ciliary or flagellar motility	6	100	66.7	4.57	1.7E-06
7129	Synapsis	9	75	88.9	4.54	2.2E-04
9988	cell-cell recognition	13	61.5	100	4.47	1.5E-07
51327	M phase of meiotic cell cycle	76	37.7	69.7	4.31	1.5E-04
7126	Meiosis	76	37.7	69.7	4.31	1.5E-04
7339	binding of sperm to zona pellucida	11	63.6	100	4.30	1.6E-07
51321	meiotic cell cycle	77	37	70.1	4.21	1.5E-04
48240	sperm capacitation	5	100	60	3.96	3.3E-05
6644	phospholipid metabolic process	127	30.1	81.1	3.90	9.7E-05
2483	antigen processing and presentation of endogenous peptide antigen	8	80	62.5	3.90	4.8E-05
19885	antigen processing and presentation of endogenous peptide antigen via MHC class I	8	80	62.5	3.90	4.8E-05
30163	protein catabolic process	220	27.2	71.8	3.85	9.4E-05

GOID	GO Name	# Genes in GO	%Genes Changed	%Genes Present	Z- Score	Parametric p-value
6643	membrane lipid metabolic process	173	28	76.3	3.77	9.0E-05
6511	ubiquitin-dependent protein catabolic process	165	28.9	69.1	3.77	6.9E-05
19941	modification-dependent protein catabolic process	167	28.4	69.5	3.65	6.9E-05
43632	modification-dependent macromolecule catabolic process	167	28.4	69.5	3.65	6.9E-05
8654	phospholipid biosynthetic process	67	33.9	83.6	3.65	3.7E-05
6352	transcription initiation	45	37.8	82.2	3.62	5.1E-05
44265	cellular macromolecule catabolic process	319	25.1	64.9	3.58	5.9E-05
6516	glycoprotein catabolic process	16	62.5	50	3.58	1.4E-04
6913	nucleocytoplasmic transport	127	29.7	71.7	3.55	1.2E-04
51603	proteolysis involved in cellular protein catabolic process	170	28	69.4	3.54	6.9E-05
6464	protein modification process	1609	19.5	78.3	3.54	8.7E-05
6508	proteolysis	806	21.3	70.5	3.49	8.2E-05
6611	protein export from nucleus	16	50	87.5	3.46	1.3E-04
9057	macromolecule catabolic process	387	23.8	67.2	3.46	7.8E-05
22414	reproductive process	248	26.1	63.3	3.46	5.4E-05
44257	cellular protein catabolic process	172	27.5	69.8	3.43	6.9E-05
46467	membrane lipid biosynthetic process	85	31.3	78.8	3.42	3.4E-05
65004	protein-DNA complex assembly	172	29.2	51.7	3.39	5.0E-05
7342	fusion of sperm to egg plasma membrane	9	66.7	66.7	3.38	8.0E-07
51169	nuclear transport	115	29.6	70.4	3.34	1.3E-04
43412	biopolymer modification	1677	19.2	78.1	3.31	8.6E-05
7017	microtubule-based process	216	25.6	72.2	3.28	9.2E-05
44267	cellular protein metabolic process	3258	18.2	74	3.26	9.3E-05
45862	positive regulation of proteolysis	5	75	80	3.21	3.1E-05
7131	meiotic recombination	17	75	23.5	3.21	6.4E-06
6003	fructose 2,6-bisphosphate metabolic process	4	75	100	3.21	2.5E-04
9894	regulation of catabolic process	19	50	63.2	3.21	2.1E-05
44260	cellular macromolecule metabolic process	3301	18.1	74.1	3.14	9.3E-05
19538	protein metabolic process	3404	18.1	74	3.12	9.2E-05

GOID	GO Name	# Genes in GO	%Genes Changed	%Genes Present	Z- Score	Parametric p-value
6986	response to unfolded protein	62	38.5	41.9	3.12	3.9E-05
51789	response to protein stimulus	62	38.5	41.9	3.12	3.9E-05
19318	hexose metabolic process	174	26.5	67.2	3.09	7.0E-05
22403	cell cycle phase	289	23.5	78.2	3.06	1.2E-04
9056	catabolic process	639	21	72.1	2.97	7.2E-05
279	M phase	232	23.9	81	2.97	1.3E-04
7340	acrosome reaction	12	57.1	58.3	2.96	4.5E-07
45026	plasma membrane fusion	10	57.1	70	2.96	8.0E-07
19883	antigen processing and presentation of endogenous antigen	11	57.1	63.6	2.96	4.8E-05
5996	monosaccharide metabolic process	178	25.8	67.4	2.93	7.0E-05
42176	regulation of protein catabolic process	14	50	71.4	2.93	2.5E-05
6515	misfolded or incompletely synthesized protein catabolic process	19	50	52.6	2.93	1.9E-04
30433	ER-associated protein catabolic process	18	50	55.6	2.93	1.9E-04
6493	protein amino acid O-linked glycosylation	16	50	62.5	2.93	1.7E-04
6367	transcription initiation from RNA polymerase II promoter	25	40	80	2.92	7.1E-05
43687	post-translational protein modification	1375	19.1	79.1	2.90	8.5E-05
51170	nuclear import	80	29.5	76.2	2.87	1.2E-04
43285	biopolymer catabolic process	285	23.2	71.2	2.78	9.5E-05
30503	regulation of cell redox homeostasis	7	60	71.4	2.68	1.8E-05
7128	meiotic prophase I	4	60	125	2.68	1.0E-07
7130	synaptonemal complex assembly	6	60	83.3	2.68	1.1E-04
51324	prophase	4	60	125	2.68	1.0E-07
6000	fructose metabolic process	13	60	38.5	2.68	2.5E-04
6072	glycerol-3-phosphate metabolic process	7	60	71.4	2.68	4.7E-05
42787	protein ubiquitination during ubiquitin-dependent protein catabolic process	5	60	100	2.68	1.2E-04
9191	ribonucleoside diphosphate catabolic process	5	60	100	2.68	1.2E-05
46470	phosphatidylcholine metabolic process	7	60	71.4	2.68	3.4E-04
31365	N-terminal protein amino acid modification	6	60	83.3	2.68	3.6E-04
6606	protein import into nucleus	79	28.8	74.7	2.68	1.2E-04

GOID	GO Name	# Genes in GO	%Genes Changed	%Genes Present	Z- Score	Parametric p-value
7001	chromosome organization and biogenesis (sensu Eukaryota)	390	22.1	64.9	2.66	1.1E-04
45582	positive regulation of T cell differentiation	11	45.5	100	2.66	1.4E-04
51168	nuclear export	40	34.6	65	2.58	2.1E-04
6096	glycolysis	76	30.2	56.6	2.54	1.8E-05
6006	glucose metabolic process	133	25.8	66.9	2.53	4.8E-05
51276	chromosome organization and biogenesis	399	21.5	66.4	2.45	1.1E-04
7018	microtubule-based movement	115	26.3	66.1	2.45	6.6E-05
18342	protein prenylation	17	41.7	70.6	2.42	1.2E-04
16311	dephosphorylation	146	24	82.9	2.38	1.4E-04
48002	antigen processing and presentation of peptide antigen	37	30.6	97.3	2.37	1.1E-04
46164	alcohol catabolic process	93	27.8	58.1	2.35	2.7E-05
8037	cell recognition	41	31.2	78	2.35	3.8E-07
6944	membrane fusion	31	37.5	51.6	2.34	8.7E-05
18346	protein amino acid prenylation	13	44.4	69.2	2.32	1.5E-04
46165	alcohol biosynthetic process	27	35	74.1	2.31	7.4E-05
46364	monosaccharide biosynthetic process	27	35	74.1	2.31	7.4E-05
19319	hexose biosynthetic process	27	35	74.1	2.31	7.4E-05
6066	alcohol metabolic process	300	21.6	72.7	2.23	7.1E-05
7049	cell cycle	762	19.4	74.4	2.22	1.1E-04
6007	glucose catabolic process	86	27.5	59.3	2.22	2.5E-05
51252	regulation of RNA metabolic process	17	38.5	76.5	2.20	3.5E-04
44248	cellular catabolic process	510	20.2	69.8	2.18	6.4E-05
32446	protein modification by small protein conjugation	75	26.2	81.3	2.17	6.3E-05
6338	chromatin remodeling	52	28.9	73.1	2.17	8.6E-05
46365	monosaccharide catabolic process	91	26.9	57.1	2.14	2.5E-05
19320	hexose catabolic process	91	26.9	57.1	2.14	2.5E-05
46916	transition metal ion homeostasis	49	30	61.2	2.08	6.1E-05
31497	chromatin assembly	142	25.4	44.4	2.03	3.6E-05
6333	chromatin assembly or disassembly	180	23.6	49.4	1.95	7.0E-05

Table 2.4
Full list of GO Terms Enriched Among Genes With Significantly Decreased Expression Through Time

GOID	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
9653	anatomical structure morphogenesis	1089	37.5	80.2	7.35	8.8E-05
9987	cellular process	12349	28.7	67	6.42	1.3E-04
32502	developmental process	3292	31.8	75.6	6.27	1.2E-04
48856	anatomical structure development	2002	33.4	74.8	6.16	9.9E-05
9058	biosynthetic process	1471	34.6	70	5.87	1.4E-04
902	cell morphogenesis	485	39.4	77.9	5.61	7.9E-05
32989	cellular structure morphogenesis	485	39.4	77.9	5.61	7.9E-05
9887	organ morphogenesis	457	39.2	82.1	5.49	8.6E-05
7167	enzyme linked receptor protein signaling pathway	278	43.7	71.6	5.42	9.7E-05
30036	actin cytoskeleton organization and biogenesis	196	46.6	74.5	5.42	7.1E-05
1525	angiogenesis	132	48.3	89.4	5.29	6.2E-05
9790	embryonic development	362	39.2	94.5	5.23	6.1E-05
30029	actin filament-based process	207	45.2	74.9	5.18	7.0E-05
7507	heart development	133	45.8	108.3	5.18	5.3E-05
51301	cell division	218	42.9	90.8	5.15	1.7E-04
35295	tube development	128	46.9	100	5.15	5.3E-05
7275	multicellular organismal development	2140	31.9	77.5	5.05	1.1E-04
48646	anatomical structure formation	173	44.4	92.5	5.04	7.4E-05
1944	vasculature development	190	42.9	93.2	4.87	7.2E-05
16043	cellular component organization and biogenesis	2656	31.4	70.2	4.86	1.2E-04
8152	metabolic process	8113	28.8	76.1	4.83	1.3E-04
44238	primary metabolic process	7338	29	75.1	4.83	1.3E-04
48731	system development	1704	32.5	74.9	4.82	1.0E-04
48514	blood vessel morphogenesis	162	44	92.6	4.78	7.5E-05
1568	blood vessel development	187	42.5	93	4.71	7.3E-05
35239	tube morphogenesis	83	48.9	106	4.68	6.2E-05

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
55008	cardiac muscle morphogenesis	4	100	200	4.67	5.5E-05
44237	cellular metabolic process	7311	28.9	75.4	4.65	1.3E-04
1763	morphogenesis of a branching structure	49	54.7	108.2	4.59	6.3E-05
9059	macromolecule biosynthetic process	899	34.8	65.5	4.48	1.5E-04
48754	branching morphogenesis of a tube	46	55.1	106.5	4.48	5.1E-05
55010	ventricular cardiac muscle morphogenesis	4	100	175	4.37	5.0E-05
7399	nervous system development	658	35.2	74.3	4.25	1.0E-04
65007	biological regulation	4497	29.6	74.5	4.21	1.2E-04
22610	biological adhesion	645	35.4	70.5	4.20	8.5E-05
7155	cell adhesion	645	35.4	70.5	4.20	8.5E-05
278	mitotic cell cycle	249	39.6	81.1	4.13	1.3E-04
7178	transmembrane receptor protein serine/threonine kinase signaling pathway	87	50	71.3	4.13	1.2E-04
15669	gas transport	17	81.8	64.7	4.12	8.7E-05
50789	regulation of biological process	4133	29.7	75.1	4.11	1.3E-04
6996	organelle organization and biogenesis	1201	33.1	65.9	4.10	1.1E-04
30865	cortical cytoskeleton organization and biogenesis	14	76.9	92.9	4.08	1.5E-04
48513	organ development	1345	32.2	76.4	4.08	1.0E-04
7067	mitosis	168	41.7	85.7	4.04	1.4E-04
87	M phase of mitotic cell cycle	170	41.5	86.5	4.04	1.4E-04
50793	regulation of developmental process	256	38.8	85.5	4.04	7.9E-05
7169	transmembrane receptor protein tyrosine kinase signaling pathway	177	42.1	75.1	4.00	8.3E-05
9880	embryonic pattern specification	41	55.3	92.7	3.96	7.9E-05
8283	cell proliferation	648	35.3	63	3.93	9.8E-05
22402	cell cycle process	646	34.9	69.2	3.92	1.4E-04
43170	macromolecule metabolic process	6411	28.8	74.7	3.91	1.3E-04
22603	regulation of anatomical structure morphogenesis	46	53.7	89.1	3.89	3.8E-05
8360	regulation of cell shape	46	53.7	89.1	3.88	3.8E-05
22604	regulation of cell morphogenesis	46	53.7	89.1	3.88	3.8E-05

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
6412	translation	656	35.2	61.4	3.87	1.4E-04
48644	muscle morphogenesis	6	80	166.7	3.80	5.5E-05
7049	cell cycle	762	33.7	74.4	3.77	1.3E-04
8150	biological process	14509	27.6	67.7	3.77	1.3E-04
30323	respiratory tube development	54	51.1	87	3.76	3.3E-05
22403	cell cycle phase	289	37.6	78.2	3.69	1.2E-04
7010	cytoskeleton organization and biogenesis	504	35.5	68.3	3.67	9.3E-05
30900	forebrain development	65	46.3	103.1	3.60	8.3E-05
31032	actomyosin structure organization and biogenesis	25	63.2	76	3.58	8.3E-05
30324	lung development	53	50	86.8	3.55	3.5E-05
9792	embryonic development ending in birth or egg hatching	149	39.2	102.7	3.48	4.8E-05
15671	oxygen transport	16	77.8	56.2	3.45	1.1E-04
43009	chordate embryonic development	147	39.1	102.7	3.42	4.9E-05
279	M phase	232	37.8	81	3.41	1.3E-04
51128	regulation of cellular component organization and biogenesis	68	46.6	85.3	3.40	9.8E-05
50794	regulation of cellular process	3752	29.3	74.9	3.39	1.2E-04
45765	regulation of angiogenesis	34	55.6	79.4	3.37	1.5E-04
8593	regulation of Notch signaling pathway	5	100	80	3.30	2.0E-05
48013	ephrin receptor signaling pathway	3	100	133.3	3.30	4.2E-05
48468	cell development	1610	30.8	74.2	3.27	1.3E-04
19538	protein metabolic process	3404	29.4	74	3.26	1.3E-04
6817	phosphate transport	84	44.6	77.4	3.25	1.2E-04
6694	steroid biosynthetic process	75	46.3	72	3.24	5.7E-05
6270	DNA replication initiation	26	64.3	53.8	3.17	1.4E-04
44249	cellular biosynthetic process	646	33.1	74.9	3.16	1.4E-04
30509	BMP signaling pathway	26	57.1	80.8	3.14	1.5E-04
6729	tetrahydropterin biosynthetic process	6	83.3	100	3.13	1.8E-04
45995	regulation of embryonic development	7	83.3	85.7	3.13	2.1E-06

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
46146	tetrahydrobiopterin metabolic process	6	83.3	100	3.13	1.8E-04
51291	protein heterooligomerization	10	83.3	60	3.13	2.8E-05
8064	regulation of actin polymerization and/or depolymerization	41	51.6	75.6	3.12	9.1E-05
30832	regulation of actin filament length	42	51.6	73.8	3.12	9.1E-05
48546	digestive tract morphogenesis	10	66.7	120	3.12	7.8E-06
51169	nuclear transport	115	42	70.4	3.09	9.8E-05
9220	pyrimidine ribonucleotide biosynthetic process	16	70	62.5	3.08	6.8E-05
6275	regulation of DNA replication	19	70	52.6	3.08	1.5E-04
30866	cortical actin cytoskeleton organization and biogenesis	11	70	90.9	3.08	1.9E-04
8088	axon cargo transport	14	70	71.4	3.08	8.9E-05
7439	ectodermal gut development	8	75	100	3.08	1.0E-05
48567	ectodermal gut morphogenesis	8	75	100	3.08	1.0E-05
44255	cellular lipid metabolic process	542	33.2	81.2	3.06	1.4E-04
32990	cell part morphogenesis	249	36.3	80.7	3.06	1.0E-04
48858	cell projection morphogenesis	249	36.3	80.7	3.06	1.0E-04
30030	cell projection organization and biogenesis	249	36.3	80.7	3.06	1.0E-04
6403	RNA localization	49	50	69.4	3.05	1.7E-04
6790	sulfur metabolic process	78	45.3	67.9	3.04	1.0E-04
7266	Rho protein signal transduction	102	41.5	80.4	3.00	1.6E-04
16125	sterol metabolic process	73	44.1	80.8	3.00	7.3E-05
1501	skeletal development	205	37.6	72.7	2.98	1.2E-04
6221	pyrimidine nucleotide biosynthetic process	22	58.8	77.3	2.98	9.7E-05
30154	cell differentiation	2098	29.9	78.6	2.97	1.2E-04
48869	cellular developmental process	2098	29.9	78.6	2.97	1.2E-04
904	cellular morphogenesis during differentiation	182	37.8	78.6	2.97	1.1E-04
48332	mesoderm morphogenesis	24	51.9	112.5	2.94	8.7E-05
43405	regulation of MAPK activity	77	44.4	70.1	2.93	9.7E-05
46164	alcohol catabolic process	93	44.4	58.1	2.93	1.3E-04

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
59	protein import into nucleus\, docking	15	60	100	2.90	1.6E-04
51261	protein depolymerization	34	50	88.2	2.87	9.3E-05
7015	actin filament organization	50	47.4	76	2.86	3.9E-05
7179	transforming growth factor beta receptor signaling pathway	57	47.4	66.7	2.86	1.1E-04
15931	nucleobase\, nucleoside\, nucleotide and nucleic acid transport	54	47.4	70.4	2.86	2.4E-04
35112	genitalia morphogenesis	1	100	300	2.86	2.3E-07
7213	acetylcholine receptor signaling\, muscarinic pathway	6	100	50	2.86	1.8E-05
48010	vascular endothelial growth factor receptor signaling pathway	2	100	150	2.86	2.0E-05
30513	positive regulation of BMP signaling pathway	3	100	100	2.86	2.5E-04
31529	ruffle organization and biogenesis	3	100	100	2.86	3.4E-04
6290	pyrimidine dimer repair	4	100	75	2.86	3.1E-05
6435	threonyl-tRNA aminoacylation	3	100	100	2.86	9.7E-05
7043	intercellular junction assembly	9	100	33.3	2.86	1.4E-06
6598	polyamine catabolic process	3	100	100	2.86	1.7E-04
30538	embryonic genitalia morphogenesis	1	100	300	2.86	2.3E-07
6695	cholesterol biosynthetic process	25	55	80	2.85	4.1E-05
46365	monosaccharide catabolic process	91	44.2	57.1	2.84	1.3E-04
19320	hexose catabolic process	91	44.2	57.1	2.84	1.3E-04
8154	actin polymerization and/or depolymerization	54	46.3	75.9	2.83	8.3E-05
50658	RNA transport	47	48.5	70.2	2.81	1.8E-04
51236	establishment of RNA localization	47	48.5	70.2	2.81	1.8E-04
50657	nucleic acid transport	47	48.5	70.2	2.81	1.8E-04
32787	monocarboxylic acid metabolic process	225	35.8	84.4	2.81	1.3E-04
46907	intracellular transport	737	32	73.7	2.81	1.4E-04
6629	lipid metabolic process	650	32.2	78.3	2.80	1.5E-04
51170	nuclear import	80	42.6	76.2	2.79	8.2E-05
35023	regulation of Rho protein signal transduction	72	42.2	88.9	2.78	1.8E-04
42127	regulation of cell proliferation	425	34	67.1	2.78	9.4E-05

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
51028	mRNA transport	43	50	65.1	2.77	2.0E-04
48568	embryonic organ development	29	50	96.6	2.77	2.8E-05
44260	cellular macromolecule metabolic process	3301	29.1	74.1	2.77	1.3E-04
44267	cellular protein metabolic process	3258	29.1	74	2.77	1.3E-04
45454	cell redox homeostasis	58	44.7	81	2.77	8.9E-05
32501	multicellular organismal process	4035	29.2	52.9	2.76	1.1E-04
9218	pyrimidine ribonucleotide metabolic process	17	63.6	64.7	2.76	6.8E-05
1676	long-chain fatty acid metabolic process	16	63.6	68.8	2.76	3.0E-04
48628	myoblast maturation	29	55.6	62.1	2.75	5.1E-05
6913	nucleocytoplasmic transport	127	39.6	71.7	2.75	9.3E-05
7163	establishment and/or maintenance of cell polarity	33	52.2	69.7	2.75	1.5E-05
7369	gastrulation	51	43.4	103.9	2.73	6.4E-05
8203	cholesterol metabolic process	67	43.4	79.1	2.73	6.0E-05
51129	negative regulation of cell organization and biogenesis	34	48.4	91.2	2.71	9.3E-05
6606	protein import into nucleus	79	42.4	74.7	2.70	8.5E-05
48557	embryonic digestive tract morphogenesis	6	66.7	150	2.70	1.4E-06
48547	gut morphogenesis	9	66.7	100	2.70	1.0E-05
30510	regulation of BMP signaling pathway	13	66.7	69.2	2.70	1.6E-04
21915	neural tube development	37	45.2	113.5	2.70	6.9E-05
43549	regulation of kinase activity	193	37	69.9	2.69	1.2E-04
48699	generation of neurons	307	34.3	81.8	2.69	9.8E-05
16050	vesicle organization and biogenesis	9	80	55.6	2.68	2.2E-04
40016	embryonic cleavage	5	80	100	2.68	9.3E-05
8215	spermine metabolic process	6	80	83.3	2.68	6.2E-06
15012	heparan sulfate proteoglycan biosynthetic process	8	80	62.5	2.68	2.5E-04
46128	purine ribonucleoside metabolic process	6	80	83.3	2.68	7.4E-05
48488	synaptic vesicle endocytosis	11	80	45.5	2.68	1.5E-04
6400	tRNA modification	7	80	71.4	2.68	1.8E-04

GOD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
30325	adrenal gland development	6	80	83.3	2.68	2.2E-04
7440	foregut morphogenesis	4	80	125	2.68	1.5E-05
1704	formation of primary germ layer	25	50	104	2.67	9.4E-05
16126	sterol biosynthetic process	31	50	83.9	2.67	7.4E-05
43406	positive regulation of MAPK activity	52	47.1	65.4	2.67	9.9E-05
47496	vesicle transport along microtubule	5	71.4	140	2.66	5.4E-05
9396	folic acid and derivative biosynthetic process	6	71.4	116.7	2.66	1.3E-04
30838	positive regulation of actin filament polymerization	7	71.4	100	2.66	2.0E-04
43525	positive regulation of neuron apoptosis	5	71.4	140	2.66	2.9E-04
7265	Ras protein signal transduction	172	37.4	71.5	2.66	1.7E-04
7409	axonogenesis	158	37.4	77.8	2.66	1.2E-04
50673	epithelial cell proliferation	23	52.4	91.3	2.65	8.4E-05
51338	regulation of transferase activity	198	36.7	70.2	2.64	1.2E-04
6007	glucose catabolic process	86	43.1	59.3	2.63	1.2E-04
22008	neurogenesis	330	33.8	82.4	2.63	1.1E-04
6461	protein complex assembly	203	36.8	65.5	2.62	1.3E-04
45859	regulation of protein kinase activity	186	36.9	69.9	2.61	1.3E-04
6631	fatty acid metabolic process	166	36.4	86.1	2.59	1.6E-04
1707	mesoderm formation	23	50	104.3	2.57	1.0E-04
96	sulfur amino acid metabolic process	21	57.1	66.7	2.56	1.1E-04
55001	muscle cell development	20	57.1	70	2.56	6.3E-05
6261	DNA-dependent DNA replication	73	43.5	63	2.55	1.2E-04
48667	neuron morphogenesis during differentiation	166	36.6	78.9	2.55	1.2E-04
48812	neurite morphogenesis	166	36.6	78.9	2.55	1.2E-04
1569	patterning of blood vessels	18	52.6	105.6	2.54	5.6E-05
51098	regulation of binding	23	52.6	82.6	2.54	1.8E-04
48627	myoblast development	30	52.6	63.3	2.54	5.1E-05
51052	regulation of DNA metabolic process	37	52.6	51.4	2.54	1.3E-04

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
8610	lipid biosynthetic process	256	34.7	78.9	2.53	1.1E-04
51726	regulation of cell cycle	461	33.5	60.3	2.52	1.3E-04
74	regulation of progression through cell cycle	459	33.5	59.9	2.51	1.3E-04
6082	organic acid metabolic process	521	32.2	79.3	2.51	1.3E-04
19752	carboxylic acid metabolic process	520	32.2	79.4	2.51	1.3E-04
8285	negative regulation of cell proliferation	189	37.3	58.2	2.48	9.9E-05
7417	central nervous system development	224	35.1	77.7	2.47	8.2E-05
16525	negative regulation of angiogenesis	15	58.3	80	2.47	1.2E-04
48565	gut development	15	58.3	80	2.47	8.9E-06
30833	regulation of actin filament polymerization	17	58.3	70.6	2.46	1.5E-04
40007	growth	252	34.5	78.2	2.46	9.9E-05
48519	negative regulation of biological process	1018	30.6	76.1	2.44	1.2E-04
10003	gastrulation (sensu Mammalia)	18	52.9	94.4	2.43	2.7E-05
46849	bone remodeling	99	38.4	86.9	2.43	1.4E-04
51216	cartilage development	37	45.5	89.2	2.42	9.3E-05
16331	morphogenesis of embryonic epithelium	52	41.5	101.9	2.42	7.3E-05
22607	cellular component assembly	536	32.6	61.8	2.41	1.4E-04
1503	ossification	89	39	86.5	2.41	1.5E-04
15980	energy derivation by oxidation of organic compounds	81	40.7	72.8	2.41	1.2E-04
2009	morphogenesis of an epithelium	106	37	101.9	2.41	1.1E-04
44272	sulfur compound biosynthetic process	35	48	71.4	2.39	1.6E-04
48523	negative regulation of cellular process	967	30.7	74.6	2.39	1.2E-04
31175	neurite development	192	35.3	79.7	2.38	1.1E-04
35088	establishment and/or maintenance of apical/basal cell polarity	12	60	83.3	2.37	2.5E-05
50818	regulation of coagulation	11	60	90.9	2.37	5.1E-05
8202	steroid metabolic process	145	36.5	79.3	2.36	9.8E-05
31214	biomineral formation	90	38.5	86.7	2.33	1.5E-04
31110	regulation of microtubule polymerization or depolymerization	14	53.3	107.1	2.32	1.2E-04

GOLD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
50678	regulation of epithelial cell proliferation	18	53.3	83.3	2.32	9.4E-05
8637	apoptotic mitochondrial	18	53.3	83.3	2.32	8.0E-05
17038	protein import	93	39.1	74.2	2.31	1.0E-04
51270	regulation of cell motility	67	40.4	85.1	2.31	6.3E-05
44275	cellular carbohydrate catabolic process	110	39.7	57.3	2.31	1.3E-04
48469	cell maturation	83	40	72.3	2.31	4.6E-05
14031	mesenchymal cell development	28	45.2	110.7	2.31	4.7E-05
6241	CTP biosynthetic process	13	62.5	61.5	2.28	6.6E-06
9209	pyrimidine ribonucleoside triphosphate biosynthetic process	13	62.5	61.5	2.28	6.6E-06
6098	pentose-phosphate shunt	9	62.5	88.9	2.28	2.4E-04
97	sulfur amino acid biosynthetic process	12	62.5	66.7	2.28	9.6E-05
1836	release of cytochrome c from mitochondria	10	62.5	80	2.28	1.3E-04
6465	signal peptide processing	8	62.5	100	2.28	3.1E-06
6740	NADPH regeneration	9	62.5	88.9	2.28	2.4E-04
10165	response to X-ray	5	62.5	160	2.28	2.1E-04
43648	dicarboxylic acid metabolic process	12	62.5	66.7	2.28	1.4E-04
50920	regulation of chemotaxis	10	62.5	80	2.28	1.8E-04
50921	positive regulation of chemotaxis	9	62.5	88.9	2.28	1.8E-04
1837	epithelial to mesenchymal transition	6	62.5	133.3	2.28	3.7E-05
9208	pyrimidine ribonucleoside triphosphate metabolic process	13	62.5	61.5	2.28	6.6E-06
46036	CTP metabolic process	13	62.5	61.5	2.28	6.6E-06
51347	positive regulation of transferase activity	118	37.5	74.6	2.27	1.2E-04
1839	neural plate morphogenesis	35	43.2	105.7	2.26	9.1E-05
7420	brain development	152	35.3	89.5	2.24	8.8E-05
6066	alcohol metabolic process	300	33.5	72.7	2.24	1.0E-04
16049	cell growth	139	36.6	72.7	2.23	6.3E-05
6096	glycolysis	76	41.9	56.6	2.23	9.1E-05
7498	mesoderm development	51	41.9	84.3	2.23	6.8E-05

GOD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
75	cell cycle checkpoint	47	46.2	55.3	2.23	1.2E-04
45333	cellular respiration	36	46.2	72.2	2.23	1.4E-04
51168	nuclear export	40	46.2	65	2.23	1.0E-04
1843	neural tube closure	22	46.2	118.2	2.23	1.0E-04
16337	cell-cell adhesion	250	34.6	63.6	2.23	6.4E-05
30182	neuron differentiation	273	33.5	78.8	2.22	1.1E-04
48562	embryonic organ morphogenesis	15	50	120	2.22	1.6E-06
30334	regulation of cell migration	58	40.8	84.5	2.22	7.2E-05
65009	regulation of a molecular function	365	32.8	71.8	2.22	1.5E-04
40012	regulation of locomotion	70	39.3	87.1	2.21	6.9E-05
42541	hemoglobin biosynthetic process	4	66.7	150	2.20	2.5E-04
20027	hemoglobin metabolic process	4	66.7	150	2.20	2.5E-04
6108	malate metabolic process	6	66.7	100	2.20	1.7E-04
48558	embryonic gut morphogenesis	5	66.7	120	2.20	2.1E-06
8156	negative regulation of DNA replication	12	66.7	50	2.20	2.5E-04
7028	cytoplasm organization and biogenesis	23	53.8	56.5	2.20	1.4E-04
15698	inorganic anion transport	153	36	72.5	2.20	1.6E-04
30239	myofibril assembly	19	53.8	68.4	2.20	5.1E-05
31111	negative regulation of microtubule polymerization or depolymerization	12	53.8	108.3	2.20	1.4E-04
55002	striated muscle cell development	19	53.8	68.4	2.20	5.1E-05
9165	nucleotide biosynthetic process	149	36.1	72.5	2.19	1.3E-04
2027	cardiac chronotropy	2	75	200	2.18	4.1E-05
9966	regulation of signal transduction	411	32.1	78.8	2.17	1.6E-04
40008	regulation of growth	169	35.1	79.3	2.17	1.0E-04
48762	mesenchymal cell differentiation	29	43.8	110.3	2.16	4.7E-05
187	activation of MAPK activity	48	43.8	66.7	2.16	9.9E-05
51246	regulation of protein metabolic process	276	33.3	77.2	2.16	1.2E-04
6284	base-excision repair	24	47.6	87.5	2.15	1.0E-04

GOD	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
7281	germ cell development	34	47.6	61.8	2.15	1.9E-05
8645	hexose transport	30	47.6	70	2.15	2.6E-04
15749	monosaccharide transport	30	47.6	70	2.15	2.6E-04
9952	anterior/posterior pattern formation	83	37.7	92.8	2.15	1.1E-04
30041	actin filament polymerization	28	47.6	75	2.15	1.2E-04
30282	bone mineralization	26	47.6	80.8	2.15	1.8E-04
16477	cell migration	285	33	80.7	2.15	9.0E-05
21700	developmental maturation	99	37.8	74.7	2.15	6.9E-05
43623	cellular protein complex assembly	48	42.9	72.9	2.14	1.1E-04
6259	DNA metabolic process	733	31	68.8	2.13	1.2E-04
6357	regulation of transcription from RNA polymerase II promoter	431	32.1	70.8	2.12	1.1E-04
31589	cell-substrate adhesion	74	38.7	83.8	2.12	6.1E-05
7219	Notch signaling pathway	51	40.9	86.3	2.11	5.6E-05
40029	regulation of gene expression\, epigenetic	64	40.9	68.8	2.11	1.9E-04
48666	neuron development	213	33.9	80.3	2.11	1.2E-04
8361	regulation of cell size	142	35.8	74.6	2.11	6.2E-05
9798	axis specification	17	50	94.1	2.09	1.4E-04
31570	DNA integrity checkpoint	26	50	61.5	2.09	4.0E-06
31109	microtubule polymerization or depolymerization	15	50	106.7	2.09	1.2E-04
45860	positive regulation of protein kinase activity	110	37	73.6	2.08	1.3E-04
7026	negative regulation of microtubule depolymerization	10	54.5	110	2.08	1.6E-04
7019	microtubule depolymerization	11	54.5	100	2.08	1.6E-04
31114	regulation of microtubule depolymerization	10	54.5	110	2.08	1.6E-04
9117	nucleotide metabolic process	229	34	70.7	2.06	1.3E-04
48771	tissue remodeling	105	36.2	89.5	2.05	1.6E-04
16052	carbohydrate catabolic process	115	37.9	57.4	2.03	1.3E-04
6220	pyrimidine nucleotide metabolic process	41	45.5	53.7	1.97	9.7E-05
271	polysaccharide biosynthetic process	24	45.5	91.7	1.97	1.4E-04

GOID	GO Name	# Genes in GO	% Genes Changed	% Genes Present	Z- Score	Parametric p-value
6793	phosphorus metabolic process	912	29.9	76.6	1.89	1.2E-04
6796	phosphate metabolic process	912	29.9	76.6	1.89	0.0001225