

Article

Expression profiles of individual human oocytes using microarray technology



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Abstract

Microarray technology is a relatively new technique that provides the investigator with the ability to monitor and quantify the expression of thousands of genes simultaneously. This technological breakthrough has the potential to provide detailed insight into cellular processes involved in the regulation of gene expression. In this study, microarray methods were used to examine the expression of linearly amplified RNA from individual and pooled ($n = 5$) human oocytes. The amplification strategy consistently produced a complex representative cDNA population. A catalogue of 1361 transcripts expressed in human oocytes was identified, of which 406 have been independently confirmed using other methods.

Keywords: gene expression, human oocytes, microarray, RNA

Introduction

The current understanding of molecular mechanisms underlying early development is still rudimentary. Further characterization of gene activity in single oocytes and embryos is urgently needed. This could provide additional insights into the regulation of oocyte maturation and preimplantation development and an improved understanding in response to external factors in the ovary, reproductive tract or artificial environment.

Historically, a variety of methodologies have been employed to examine gene expression such as C_o_t value assays (Davidson and Hough, 1969), northern gels (Thomas, 1980) and dot- or slot-blots (White and Bancroft, 1982). However, the poor sensitivity of these techniques, coupled with the scarcity of human oocytes and embryos available for research, had placed severe limitations on investigations of gene activity during early human development. In particular, the quantification of mRNA transcript number in individual oocytes or embryos was not possible using these techniques.

The advent of reverse transcription–polymerase chain reaction (RT-PCR) (Rappolee *et al.*, 1988) has largely overcome obstacles posed by the shortage of material available for

analysis. Nonetheless, the gene-by-gene analysis that this method allows generally provides too narrow a view of underlying regulatory networks to further the understanding of complex physiological pathways. Although adequate for the quantification of a single transcript, the analysis of multiple genes using RT-PCR is extremely laborious. Furthermore, the amount of material remains limiting, even for RT-PCR based analyses. The most detailed RT-PCR study of single oocytes and embryos reported thus far was only capable of quantifying nine genes per oocyte/embryo, leaving over 99.97% of the genes in the genome untested (Wells *et al.*, 2002).

The need to ‘cast a wider net’ in a variety of biological applications has prompted the development of microarray techniques (Schenna *et al.*, 1995; Fodor *et al.*, 1991). Microarrays are a relatively new molecular tool, which provide the researcher with a genome-wide perspective by profiling the expression of thousands of genes in a single experiment. Unprecedented amounts of information are obtained enabling massive parallel mining of biological data. In addition, microarray methods produce valuable quantitative information (Taniguchi *et al.*, 2001). The ability to analyse and quantify the expression of multiple genes simultaneously would create an important avenue towards understanding

gamete function and the molecular events coordinating early development. Unfortunately, typical microarray protocols require the isolation of considerable amounts of mRNA (~5 µg), several orders of magnitude greater than that contained within a single oocyte or embryo (Lockhart *et al.*, 1996; Shalon *et al.*, 1996). Clearly some form of RNA amplification will be required if microarrays are to be applied to the analysis of single cells.

Recently, protocols have been described for the linear amplification of broad classes of mRNAs (Eberwine 1996; Craig *et al.*, 1997; Kacharmina *et al.*, 1999). These methods utilize an in-vitro transcription reaction from a highly specific T7 RNA polymerase promoter producing a complex, representative population of cDNA fragments. This approach circumvents many of the problems inherent with the most commonly employed amplification strategies that depend on PCR. Namely, PCR-based techniques typically suffer from low fidelity and mis-incorporation of nucleotides resulting from the use of *Taq* DNA polymerase, as well as a bias towards the amplification of shorter sequences. Thus, with the use of linearly amplified RNA methods, the primary obstacle to single cell microarray analysis can potentially be overcome.

This study sought to determine whether RNA from individual human oocytes could be linearly amplified to sufficient levels to produce an informative expression profile using microarray methods. The study also set out to establish whether the techniques employed would yield reliable and reproducible results. Results obtained from individual samples (i.e. single oocytes) were contrasted with those produced from pooled material (five or more oocytes) to ascertain whether they were comparable. Finally, the study verified the accuracy of the microarray data by comparing them with previous studies of gene expression in human oocytes. These studies employed various methodologies and examined a wide variety of genes, thus providing an extensive independent data set with which to assess the validity of the microarray results.

Materials and methods

In keeping with minimum information about a microarray experiment (MIAME; Brazma *et al.*, 2001) regarding data reporting conventions for microarray experiments, the following are detailed descriptions of the samples and protocols used throughout this study.

Oocytes and embryos

Spare human oocytes were obtained from patients undergoing assisted reproduction at the Institute for Reproductive Medicine and Science of Saint Barnabas following written consent and Institutional Review Board approval. Oocytes used in this study ($n = 14$) consisted of discarded mature oocytes (metaphase II) that failed to fertilize following conventional in-vitro insemination. While it is possible that these oocytes may still retain cytoplasmic spermatozoa that have failed to decondense (Bedford and Kim, 1993), this is not a concern in this study in which cytoplasmic mRNA is the object of analysis rather than nuclear or cytoplasmic DNA.

Isolation of total RNA

Individual oocytes were briefly incubated in acidified Tyrode's solution (Sigma, St Louis, MO, USA) to remove the zona pellucida. Afterwards they were washed in PBS (Mg²⁺, Ca²⁺-free)/PVA 0.1% with RNasin inhibitor (Promega, Madison, WI, USA) and flash frozen at -70°C pending further processing. RNA was isolated using Arcturus' PicoPure™ RNA Isolation Kit according to the manufacturer's instructions (Arcturus, Mountain View, CA, USA).

RNA amplification and target preparation

Using the Arcturus' RiboAMP™ RNA Amplification Kit, RNA was amplified. RNA transcript labelling was performed using ENZO's BioArray High Yield RNA Transcript Labeling Kit T7 (ENZO Diagnostics, Inc., Farmingdale, NY, USA). The resultant labelled cRNA (complementary RNA) was then purified and fragmented by the addition of ×5 fragmentation buffer (GeneChip® Sample Cleanup Module; Affymetrix Inc., Santa Clara, CA, USA) as per manufacturer's recommendations. Gel analysis was performed to verify the presence of a distribution of RNA fragment sizes ranging from 35 to 200 bases (**Figure 1**).

Target hybridization and probe array scanning

Prior to hybridization to an Affymetrix GeneChip® Human Genome Focus Array (part number 900377; Affymetrix), a hybridization cocktail was prepared consisting of the fragmented cRNA, probe array controls (eukaryotic hybridization controls bioB, bioC, bioD, cre, at a final concentration of 1.5, 5, 25 and 100 pmol/l respectively; control oligonucleotide B2, 50 pmol/l), acetylated BSA 0.5 mg/ml, ×2 hybridization buffer (final ×1 concentration is 100 mmol/l MES, 1 mol/l [Na⁺], 20 mmol/l EDTA, 0.01% Tween 20), and herring sperm DNA 0.1 mg/ml. The target was then hybridized to a GeneChip® human genome focus array representing over 8500 expressed human sequences. A complete list of the probe sets interrogated by this array can be found at Affymetrix's NetAffx™ Analysis centre (<https://www.affymetrix.com/site/login/login.affx>). Hybridization occurred during a 16-h incubation period at 45°C while under a constant rotation of 60 rpm in a GeneChip 640 hybridization oven. Following hybridization, the probe array was washed and stained in an automated GeneChip 400 fluidics station. The probe array was then scanned using a GeneChip scanner. Data were analysed using Affymetrix® Microarray Suite 4.0 software.

Statistics

To determine if a relationship existed between the expression results obtained from different oocyte samples, Pearson and Spearman correlation analysis was used. Statistical analysis was performed using WINKS Basic Edition, version 4.5 (TexaSoft, Cedar Hill, TX, USA).

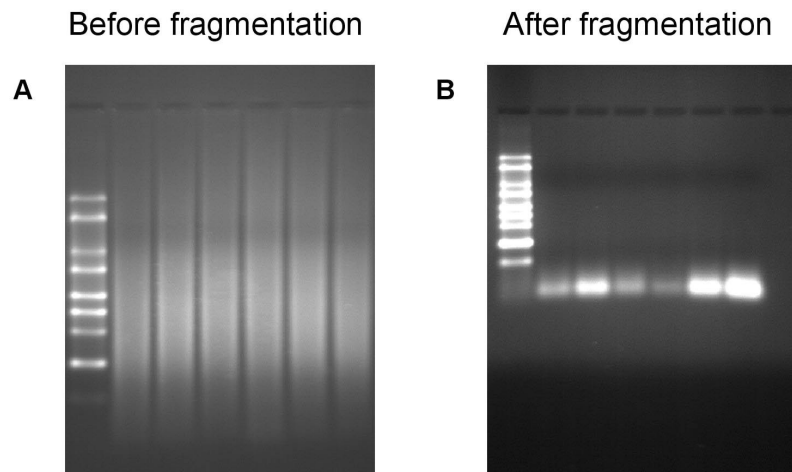


Figure 1. Agarose gel electrophoresis of amplified oocyte RNA both before (A) and after (B) fragmentation. The molecular weight marker used is transcript RNA marker 0.2–10 kB (R-7020; Sigma, St Louis, MO, USA).

Results

A variety of controls are incorporated into the design of Affymetrix GeneChips in order to monitor and troubleshoot potential problems during sample preparation and processing. These include probes specific for housekeeping gene transcripts used to evaluate the integrity of the input cDNA. Staggered concentrations of prokaryotic gene transcripts are added directly to the hybridization cocktail, in order to assess the performance of the hybridization process as well as to monitor subsequent washing and staining steps. Parameters such as per cent present calls (the percentage of gene transcripts scored as present in the sample), background and noise checks were evaluated. The results obtained for samples included in this study were consistent with those deemed satisfactory for single cell preparations based on optimization experiments, namely, average present calls >25%, average background <100, and average noise <2.

Signal intensities obtained from amplified individual oocytes were plotted against one another in order to determine the degree of variability between them. A total of four single oocytes were amplified and compared with each other in every possible combination. One of the scatterplots produced is presented in **Figure 2**. Each data point represents the results for one of the >8500 expressed sequences analysed, indicative of the number of transcripts present in the oocyte. The average Pearson's correlation coefficient calculated was 0.95 ± 0.02 with a P -value ≤ 0.001 , demonstrating that the vast majority of genes assessed had similar levels of expression between samples.

In order to confirm whether the genes scored as 'present' using this microarray approach are indeed expressed in human oocytes, the results were compared with previously published oocyte gene expression data obtained in this study and others using a variety of methodologies (Stanton and Green, 2001; Steuerwald *et al.*, 2001; Metcalfe *et al.*, 2003). These earlier studies examined either human material or the expression of well-matched human homologues in mouse material. The list of 406 genes whose expression was validated by this means is presented in **Table 1** (pp. 329–336), subdivided by function.

Average signal values obtained for these genes are given as well. In some instances, the transcript assessed using the microarray was not identical to that previously reported, but was derived from a closely related isoform instead.

Table 2 (p. 336) presents summary information describing the expression profiles of amplified single oocyte samples ($n = 4$) versus that of pools of five oocytes ($n = 2$). The total number of gene transcripts identified as present in all individual oocytes was 1467, whereas 1823 were found in both replicates of pooled material. Both groups had 1361 gene products in common (detection P -values ≤ 0.05). Single oocytes expressed 106 genes that were not found in the pooled material while the pooled material expressed 462 genes exclusively. This is possibly the result of the small sample study, individual variations in expression and the consequence of this type of single cell technology.

Discussion

This study optimized and applied RNA amplification techniques that generate sufficient material to permit analysis of single oocytes via microarrays. Using this approach, 1361 transcripts were identified that were consistently expressed in all oocytes tested (detection P -values ≤ 0.05). The presence of these transcripts suggests specific pathways that may be active in the human oocyte. Conversely, the 7432 transcripts that were absent from all of the samples indicate pathways that are not used by MII oocytes. Although it should be noted that altered ovarian stimulation regimens, deviations in in-vitro conditions and the selection criteria used for obtaining the samples could elicit the activation of genes and pathways not observed in this study.

The application of new technology for genetic analysis requires careful validation to ensure accuracy, particularly when used at the single cell level. The results from the reproducibility analysis demonstrated that the amplification strategy employed in this study produces expression data sets with a high degree of concordance between samples. The signal strengths attained for transcripts scored as 'present' in all amplified samples were closely related to each other,

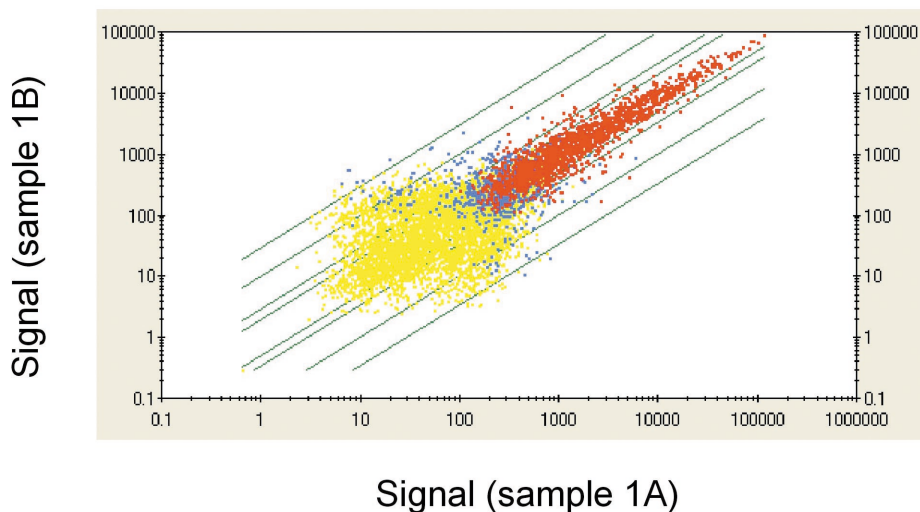


Figure 2. Scattergraph plot comparing the expression of an individual oocyte sample to another following microarray analysis. A total of 8793 gene products were examined simultaneously. Red dots represent transcripts present in both samples, yellow dots indicate transcripts absent in both, and blue dots represent presence in one and absence in the other. The innermost vertical pair of lines indicates transcripts differentially expressed at 2-fold levels. Likewise, the additional pairs of lines represent 3-, 10- and 30-fold changes respectively. Note that the vast majority of transcripts present in both oocytes (red dots) lie within the innermost lines.

suggesting that the amplification process had not introduced a noteworthy bias. On average a little over 100 genes were uniquely expressed by individual oocytes. This minimal variability in expression (affecting ~1% of the genes assessed) is likely due to differences in the genetic background of the patients from whom oocytes were derived, patient-specific responses to ovarian stimulation, and altered gene expression due to subtle differences in the microenvironment of each oocyte. It may have also been the consequence of the alternate modes by which oocytes exposed to spermatozoa *in vitro* fail to proceed to sperm decondensation. The fact that variation between samples was small and that many of the transcripts, including those involved in apoptosis, cell cycle regulation, signalling pathways and protein synthesis, detected during this study had also been identified by previous research, leads to the conclusion that the amplified material is representative of the original samples.

An increase in the total number of gene transcripts identified as present was noted in pooled material relative to single oocytes. Such an observation is expected as pooled oocytes are drawn from several patients with differences in their response to treatment, genetic background and cause of infertility. Thus, pooled samples are expected to display increased genetic complexity. However, the pooled samples did not show a marked increase in expression profiles, indicating the reliability of the methods employed.

The expression analysis results obtained in this study closely parallel those reported by others using conventional techniques such as RT-PCR to investigate individual genes. Evidence for this is given by the substantial number of transcripts identified in this study that have previously been shown to be present in oocytes (Stanton and Green, 2001; Steuerwald *et al.*, 2001; Metcalfe *et al.*, 2003). Of the 1361 transcripts that were present in all of the oocytes analysed, independent data were available for 406. It is remarkable that

in every case, the previously published results were concordant with the present findings using microarrays. The 406 validated genes cover a broad spectrum of biological functions, from those regulating the nuclear apparatus through those involved in energy metabolism and protein biosynthesis. Admittedly, a considerable number of genes remain unconfirmed. Still, given a data set of the monumental proportion presented here and the diverse methodologies employed during previous studies, it is indeed astounding that the expression of several hundred genes could be confirmed.

The results described in this investigation highlight the potential of microarray technology in reproductive medicine. Parallel analysis of unprecedented numbers of gamete transcripts promises to provide a detailed insight into the role that these messages play during early human development. The experiments reported here have allowed the assembly of a large catalogue of genes, now known to be expressed in human MII oocytes. This catalogue can be interrogated in a variety of ways. For example if a particular gene is shown to be of importance in the oocytes of a different species (e.g. *Xenopus*), the catalogue can be checked for the presence of human homologue(s), thus providing an indication of whether the gene is also of functional significance in humans.

Knowledge of the genes expressed during specific developmental stages should allow a new understanding of the signalling networks regulating gametogenesis and embryogenesis, providing a global view of these processes as well as a first glimpse into key areas of embryonic health and viability. Thus, it follows that microarray analysis could be used to screen for clinically useful reproductive markers. Once identified, genes informative as regards oocyte and/or embryo competence could form the basis of new preimplantation genetic diagnostic tests, assisting the identification and transfer of the most viable embryos during assisted reproductive treatments and potentially revolutionizing the practice of IVF.

Table 1. Single oocyte samples independently confirmed gene expression.

<i>Gene symbol/description</i>	<i>Average signal</i>
<i>Apoptosis</i>	
BECN1 beclin 1 (coiled-coil, myosin-like BCL2-interacting protein)	689.38
BNIP3 BCL2/adenovirus E1B 19 kD-interacting protein 3	2467.63
PDCD6 programmed cell death 6	1231
API5L1 ^a apoptosis inhibitor 5	853.02
<i>Cell cycle</i>	
CUL1 cullin 1	1755.63
PTTG1 pituitary tumour-transforming 1	50,318.83
FOXO1A forkhead box O1A (rhabdomyosarcoma)	771.67
SMC1 ^a SMC1 (structural maintenance of chromosomes 1, yeast)-like 1	647.15
KIAA0165 extra spindle poles, <i>S. cerevisiae</i>	2657.4
TP53BP2 ^a tumour protein p53-binding protein, 1	625.42
CKS1 CDC28 protein kinase 1	4113.02
MCM2 minichromosome maintenance deficient (<i>S. cerevisiae</i>) 2 (mitotin)	2440.3
FANCC Fanconi anemia, complementation group c	982.02
SAM68 GAP-associated tyrosine phosphoprotein p62 (Sam68)	3409.02
RANBPM novel centrosomal protein RanBPM	23,542.52
PCM1 pericentriolar material 1	1463.7
CKS2 CDC protein kinase 2	2092.5
BTG1 ^a BTG family, member 1	355.88
MCM6 minichromosome maintenance deficient (mis5, <i>S. pombe</i>) 6	2519.73
MAD2L1 ^a MAD2 (mitotic arrest deficient, yeast, homologue)	11,171.55
<i>Circadian rhythms</i>	
CSNK2A2 ^a casein kinase	972.63
<i>Cytoskeleton</i>	
CTNNB1 catenin (cadherin-associated protein), beta 1 (88 kD)	496.15
CNN3 calponin 3, acidic	1033.1
WDR1 WD repeat domain 1	4314.25
CFL1 cofilin 1 (non-muscle)	1753.65
CLTA clathrin, light polypeptide (Lca)	2057.15
CAPZA1 ^a capping protein (actin filament) muscle Z-line, alpha 1	1625.47
VIL2 villin 2 (ezrin)	502.27
PTPN13 protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)	295.92
KIF3A ^a kinesin heavy chain member 3A	2659.6
KIF3B ^a kinesin heavy chain member 3B	633.45
KIF4A ^a kinesin heavy chain member 4A	1589.9
KIF13B ^a kinesin family member 13B	1002.1
KIF5B ^a kinesin family member 5B	19,437.78
PLS1 plastin 1 (I isoform)	8851.5
<i>Secretory pathways, exocytosis, endocytosis, etc.</i>	
PRDX3 ^a peroxiredoxin 3	12,505.85
TM9SF2 transmembrane 9 superfamily member 2	1273.75
SYPL synaptophysin-like protein	5543.13
RAB6A ^a RAB6A, member RAS oncogene family	1212.75
STAU stauflen (RNA binding protein)	3872.2
PEX12 ^a peroxisome biogenesis factor 12	703.17
PEX3 ^a peroxisome biogenesis factor 3	607.13
SNAP29 ^a synaptosomal-associated protein, 29 kD	469.15
CTSL ^a cathepsin L	1819.75
ATP6B2 ^a ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump) beta polypeptide, 5658 kD, isoform 2	969.38
RAB3GAP RAB3 GTPase-activating protein	1538.15
SCAMP2 ^a secretory carrier membrane protein 2	699.42

Table 1 contd

RAB27A ^a member RAS oncogene family	574.35
RAB5A ^a member RAS oncogene family	1572.32
RAB2 member RAS oncogene family	766.05
RAB22A ^a member RAS oncogene family	2355.1
RAP1A ^a member of RAS oncogene family	1134.28
LOC51138 ^a COP9 complex subunit 4	2291.5
SNX2 ^a sorting nexin 2	1415.52
SNX16 ^a sorting nexin 16	340.9
SEC23 ^a SEC23 (<i>S. cerevisiae</i>)	889.85
SEC14 ^a SEC14 (<i>S. cerevisiae</i>)	2462.57
VATd ^a ATPase, vacuolar	2465.63
ARF4 ADP-ribosylation factor 4	3234.2
SYBL1 synaptobrevin-like 1	21,781.47
ATPJ ^a ATPase, H ⁺ transporting, lysosomal (vacuolar proton pump), member J	4007.1
PLEK2 pleckstrin 2 (mouse) homologue	4396.88
VAMP VAMP (vesicle-associated membrane protein)-associated protein	5324.72
ARF6 ADP-ribosylation factor 6	458.3
STX8 ^a syntaxin 8	1610.15
SYNJ1 synaptojanin	2279.23
BIG2 brefeldin A-inhibited guanine nucleotide-exchange protein 2	467.92
<i>Kinases</i>	
AK5 ^a adenylate kinase 5	1212.7
STK18 serine/threonine kinase	969.15
LOC51727 UMP-CMP kinase	1032.72
PGK1 phosphoglycerate kinase1	7726.13
CSNK1G2 casein kinase 1, gamma 2	728.3
STK1 ^a serine/threonine kinase 1	18,941.45
STK3 ^a serine/threonine kinase 3 (Ste20, yeast homologue)	1545.13
STK6 ^a serine/threonine kinase 6	59,721.72
CAMK2G calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	1542.93
<i>Membrane receptors, ion channels, etc.</i>	
TPD52L2 tumour protein D52-like 2	1182.32
ITGA10 ^a integrin, alpha 10	625.3
ITGAE ^a integrin, alpha E	12,855.77
FAT FAT tumour suppressor (<i>Drosophila</i>) homologue	1944.5
EPB41L2 erythrocyte membrane protein band 4.1-like 2	1838.23
CD36L2 ^a CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2	832.83
SLC7A9 ^a solute carrier family 7 (cationic amino acid transporter, y+ system), member 9	59,721.72
SLC7A7 ^a solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	2031.18
SLC25A13 ^a solute carrier family 25, member 13 (citrin)	2504.4
SLC18A2 ^a solute carrier family 18 (vesicular monoamine), member 2	421
SLC25A5 ^a solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5, nuclear gene encoding mitochondrial protein	16,368.8
SLC4A7 ^a solute carrier family 4, sodium bicarbonate cotransporter, member 7	459.17
SLC12A5 ^a solute carrier family 12, (potassium-chloride transporter) member 5	7109.63
SLC11A2 ^a solute carrier family 11 (proton-coupled divalent metal ion transporters) member 2	1066.75,
SLC5A3 ^a solute carrier family 5 (inositol transporters), member 3	3475.93
SLC35A2 ^a solute carrier family 35 (UDP-galactose transporter), member 2	996.9
SLC9A6 ^a solute carrier family 9 (sodium hydrogen exchanger), isoform 6	774.08
SLC21A11 ^a solute carrier family 21 (organic anion transporter), member 11	3437.13
SLC25A14 ^a solute carrier family 25 (mitochondrial carrier, brain), member 14, transcript variant long, nuclear gene encoding mitochondrial protein	4663.67
SLC35A3 ^a solute carrier family 35 (UDP-N-acetylglucosamine (UDP-GlcNAc) transporter), member 3	448.7
SLC25A3 ^a solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3, nuclear gene encoding mitochondrial protein, transcript variant 1b	3201.38
PTPRK ^a protein tyrosine phosphatase, receptor type, K	2257.9
PTPRM ^a protein tyrosine phosphatase, receptor type, M	605.97

Table 1 contd

PTPRG ^a protein tyrosine phosphatase, receptor type, G	1423.13
PTPRD ^a protein tyrosine phosphatase, receptor type, D	741.72
PTPN1 ^a protein tyrosine phosphatase, non-receptor type 1	764.4
PSEN2 presenilin 2 (Alzheimer disease 4)	1337.7
ATP1B3 ^a ATPase, Na ⁺ /K ⁺ transporting, beta 3 polypeptide	1644.05
SLC23A12 ^a solute carrier family 25 (nucleobase transporters), member 12	648.15
CLCN3 chloride channel 3	1219.05
ATP2A2 ATPase Ca ²⁺ transporting, cardiac muscle, slow twitch 2	7908.6
TMEM2 ^a transmembrane protein 2	1073.38
CD81 CD81 antigen (target of antiproliferative antibody 1)	303.15
TM9SF2 ^a transmembrane 9 superfamily member 2	1273.75
TM7SF1 ^a transmembrane 7 superfamily member 1	6842.58
EFNB2 ephrin-B2	4356.65
SLC12A2 solute carrier family 12 (sodium/potassium/chloride transporter), member 2	1400.3
KIT v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue	2129.55
LDLR ^a low density lipoprotein receptor	620.75
CLDN10 ^a claudin 10	636.48
KCNN4 potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	2224.48
TRPC3 ^a transient receptor potential channel 3	820.88
SLC3A1 solute carrier family (cystine, dibasic and neutral amino acid transporter, activator of cystine, dibasic and neutral amino acid transport) member 1	2348.2
SLC35A1 solute carrier family 35 (CMP-sialic acid transporter) member 1	1751.15
NET-6 ^a tetraspan NET 6	658.92
MMP1 ^a matrix metalloproteinase 1	859.05
ABCD3 ^a ATP-binding cassette, sub-family D (CFTR/MRP), member 3	13,018.95
LOC51123 NY-REN-45 antigen	13,024.35
<i>Mitochondria</i>	
COX6C cytochrome c oxidase subunit VIc	546.5
CYP24 ^a cytochrome P450, subfamily XXIV	1318.1
ETFA ^a electron-transfer-flavoprotein, alpha polypeptide (glutamic aciduria II)	5227.33
TIM17 translocase of inner mitochondrial membrane 17 (yeast) homologue A	6232.42
SDHC succinate dehydrogenase complex, subunit c, integral membrane protein, 15 kD	883.53
COX7A2 ^a 2 cytochrome c oxidase subunit VIIa2	3294.98
MTIF2 mitochondrial translational initiation factor 2	4068.63
ATP5JG ATPsynthase, H ⁺ transporting, mitochondrial F1F0, subunit g	2424.52
TOMM20 ^a translocase of outer mitochondrial membrane 20 (yeast)	751.05
TOMM34 ^a translocase of outer mitochondrial membrane 34 (yeast)	1294.2
TCF6L1 transcription factor 6-like 1 (mitochondrial transcription factor 1-like)	585.47
<i>Structural nuclear proteins</i>	
NPM1 nucleophosmin (nucleolar phosphoprotein B23, numatrin)	13,975.07
NUP88 nucleoporin 88 kD	1098.15
LAMB1 laminin, beta 1	4231.6
NCL nucleolin	1523.97
PCNA proliferating cell nuclear antigen	23,150.22
HNRPK ^a 5 heterogeneous nuclear ribonucleoprotein K	1819.43
HNRPD ^a heterogeneous nuclear ribonucleoprotein D	1541.48
HNRPR ^a heterogeneous nuclear ribonucleoprotein R	4742.67
HNRPC ^a heterogeneous nuclear ribonucleoprotein C	1564.73
HNRPA0 ^a heterogeneous nuclear ribonucleoprotein A0	855.55
NUP54 nucleoporin p54	1578.23
NOP56 ^a nucleolar protein NOP56	345.38
NUP153 nucleoporin 153 kD	8195.4
NASP nuclear autoantigenic sperm protein (histone-binding)	598.28
PP15 nuclear transport factor 2 (placental protein 15)	576.58
DKC1 dyskeratonin congenita 1 dyskenin	4277.35
UBN1 ubinuclein 1	3367.25

Table 1 contd

Phosphatases

PTPCAAX2 protein tyrosine phosphatase type IVA, member 2	2280.32
MKP-L MKP1-like protein tyrosine phosphatase	3284.88
CDC25B ^a cdc25B	2370.18
PPP1CC protein phosphatase 1, catalytic subunit, gamma isoform	4525.35
PPM1A protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha isoform	4290.98
PTPRG ^a protein tyrosine phosphatase, receptor type, G	1423.13
PTPRM ^a protein tyrosine phosphatase, receptor type, M	605.97
PTPRK ^a protein tyrosine phosphatase, receptor type, K	2257.9
PTPRD ^a protein tyrosine phosphatase, receptor type, D	741.72
PTPN1 ^a protein tyrosine phosphatase, non-receptor type 1	295.92
PTPN13 protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)	295.92
PTPN3 ^a protein tyrosine phosphatase, non-receptor type 3	468.7
PPP2R2A protein phosphatase 2 (formerly 2A), regulatory subunit B (PR52), alpha isoform	2657.07
PPP2R5E ^a protein phosphatase 2, regulatory subunit B (B56), epsilon isoform	1276.97
PPP2R5C ^a protein phosphatase 2, regulatory subunit B (B56), gamma isoform	2292.13
MYPT1 myosin phosphatase, target subunit 1	6127.67
PPM1D protein phosphatase 1D magnesium-dependent, delta isoform	1242.72

Protein degradation

UBE2D3 ubiquitin conjugating enzyme E2D 3 (homologous to yeast UBC4/5)	4101.25
CUL1 cullin 1	1755.63
UBE2D2 ubiquitin conjugating enzyme E2D2 (homologous to yeast UBC4/5)	3388.07
USP9X ubiquitin specific protease 9, X chromosome (<i>Drosophila</i> fat facets related)	6746.9
USP7 ^a ubiquitin specific protease 7 (herpes virus-associated)	13,011.02
USP13 ^a ubiquitin specific protease 13 (isopeptidase T-3)	721.65
USP22 ^a ubiquitin specific protease 22	579.42
USP16 ^a ubiquitin specific protease 16	3669.62
USP25 ^a ubiquitin specific protease 25	10,729.5
FBXO7 ^a F-box only protein	1978.83
UBE2H ubiquitin-conjugating enzyme E2H (homologous to yeast UBC8)	4160
FBXW2 ^a f-box and WD-40 domain protein 2	384.48
USP1 ubiquitin specific protease 1	27,648.7
UBE2A ubiquitin-conjugating enzyme E2A (RAD 6 homologue)	5449.88
HSPF1 heat shock 40 kD protein 1	447
PSMB4 ^a proteasome (prosome, macropain) subunit, beta type, 4	2771.8
CUL3 cullin 3	7752.85
USP14 ubiquitin-specific protease 14 (tRNA-guanine transglycosylase)	4670.92
UBE3A ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)	874.7

Protein synthesis

EIF2B1 eukaryotic translation initiation factor 2B, subunit (alpha, 26 kD)	577.17
RPL17 ribosomal protein L17	1625.95
RPS3A ribosomal protein S3A	12,234.35
EIF4G2 eukaryotic translation initiation factor 4 gamma, 2	2969.7
RPL24 ribosomal protein L24	1937
EIF4A2 eukaryotic translation initiation factor 4A, isoform 2	42,321.95
RPL7A ribosomal protein L7a	4731.03
EIF3S10 ^a eukaryotic translation initiation factor 3, subunit 10 (theta, 150,170 kD)	1698.2
EIF3S3 ^a eukaryotic translation initiation factor 3, subunit 3 (gamma, 40 kD)	1761.5
EIF3S5 ^a eukaryotic translation initiation factor 3, subunit 5 (epsilon, 47 kD)	2311.32
NARS asparaginyl-tRNA synthetase	1158.5
RPL5 ribosomal protein L5	1301.47
RPL13A ribosomal protein L13a	842.2
RPL6 ribosomal protein L6	1737.4
RPL9 ribosomal protein L9	9671.33
RPLP1 ^a ribosomal protein, large P1	1260.85
RPL27 ribosomal protein L27	3626.05

Table 1 contd

RPS10 ribosomal protein S10	1960.05
<i>Secreted proteins</i>	
TGF α^a transforming growth factor, alpha	622.58
MFAP1 microfibrillar-associated protein 1	1103.35
COL4A3 ^a collagen, type V alpha 3	4455.63
<i>Signalling pathways</i>	
RBBP7 ^a retinoblastoma-binding protein 7	20,752.95
RGS2 regulator of G protein signalling 2, 24 kD	45,764.55
TRIP12 thyroid hormone receptor interactor 12	1558.88
TRIP13 ^a thyroid hormone receptor interactor 13	2517.38
TRIP15 ^a thyroid receptor interacting protein 15	6473.48
STK15 ^a serine/threonine kinase	18,307.25
PARG1 PTPL1-associated RhoGAP1	582.1
LOC51306 GAP-like protein	3675.7
SSH3BP1 spectrin SH3 domain binding protein 1	650.8
YES1 3 v-yes-1 Yamaguchi sarcoma viral oncogene homologue 1	11,851.4
UBL1 ubiquitin-like 1 (sentrin)	336.45
TNFAIP1 tumour necrosis factor alpha-induced protein 1 (endothelial)	975.05
DKFZP434L1021 potassium channel modulatory factor	7704.82
STAT1 ^a signal transducer and activator of transcription 1	711.75
PPP2R2A protein phosphatase 2 (formerly 2A), regulatory subunit B (PR52), alpha isoform	2657.07
CSNK1G3 ^a casein kinase 1, gamma 3	2831.02
NR1H4 ^a nuclear receptor family 1, group H, member 4	770.25
PTPN13 protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)	295.92
TRAF6 ^a TNF receptor-associated factor 4	3036.38
MAPK6 ^a mitogen-activated protein kinase 6	5336.78
GNG5 ^a guanine nucleotide binding protein (G protein), gamma 5	1337.42
PDZK1 PDZ domain-containing 1	755.9
GDI2 ^a GDP dissociation inhibitor 2	3043.88
PRKCI ^a protein kinase C, iota	1446.88
CAMK2G calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	1542.93
IQGAP2 IQ motif containing GTPase activating protein 2	986.17
<i>DNA, chromatin</i>	
H3F3B H3 histone, family 3B (H3.3B)	56,092.2
TOP1 topoisomerase (DNA)1	2824.52
HMG14 ^a high-mobility group (non-histone chromosomal) protein 14	39,031.85
NAP1L1 nucleosome assembly protein 1-like 1	3264.33
DNMT1 DNA (cytosine-5-)-methyltransferase 1	4425.48
KIAA0026 MORF-related gene X	5090.4
H2AFX ^a H2A histone family, member X	491.53
SMARCA5 SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	731.78
H1F0 H1 histone family, member 0	2986
NHP2L17 ^a non-histone chromosome protein 2 (<i>S. cerevisiae</i>)-like 17	4631.2
HMG4 high-mobility group (non-histone chromosomal) protein 4	3773.08
DNMT3A DNA (cytosine-5-)-methyl transferase 3 alpha	823.52
NASP nuclear autoantigenic sperm protein (histone-binding)	598.28
POLE2 polymerase (DNA directed), epsilon 2	2269.9
HBOA histone acetyltransferase	827.63
NCBP2 nuclear cap binding protein subunit 2, 20 kD	1523.53
ATRX alpha thalassaemia/mental retardation syndrome X-linked (RAD54 (<i>S. cerevisiae</i>) homologue)	3634.13
<i>RNA, transcription</i>	
KIAA0660 Ras GTP-ase activating protein SH3 domain-binding protein 2	1235.38
NCOA1 ^a nuclear receptor coactivator 1	636.55
NCOA2 ^a nuclear receptor coactivator 2	4135.67

Table 1 contd

NCOA3 ^a nuclear receptor coactivator 3	6968
DDX3 DEAD/H (Asp–Glu–Ala–Asp/His) box polypeptide 3	14,553.1
GTF2B general transcription factor IIB	16,289.48
HNRPK heterogeneous nuclear ribonucleoprotein K	1819.43
E2F1 ^a E2F transcription factor 1, p130-binding homologue	591.38
PABPC4 poly(A)-binding protein, cytoplasmic 4 (inducible form)	1778.93
MSH6 mutS (<i>E. coli</i>) homologue 6	1962.68
CRSP3 ^a cofactor required for Sp1 transcriptional activation, subunit 3	641.97
BTF3 basic transcription factor 3	908.68
ZNF9 zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)	8887.6
DDX5 DEAD/H (Asp–Glu–Ala–Asp/His) box polypeptide 5 (RNA helicase, 68 kD)	8334.3
TCEB1L transcription elongation factor B (SIII), polypeptide 1-like	933.55
ORC5L origin recognition complex, subunit 5 (yeast homologue)-like	2963.32
ZNF216 zinc finger protein 216	33,022.13
SFRS10 ^a splicing factor, arginine/serine-rich 3	8348.13
SFRS4 ^a splicing factor, arginine/serine-rich 4	852.3
SFRS11 ^a splicing factor, arginine/serine-rich 11	7846.23
HNRPA1 heterogeneous nuclear ribonucleoprotein A1	31,336.28
KHSRP KH-type splicing regulatory protein (FUSE binding protein 2)	1393.35
RBM4 ^a RNA binding motif protein 4	4231.23
TAF2N ^a TATA box binding protein (TBP)-associated factor, RNA polymerase II, N	440.68
SNRPD3 ^a small nuclear ribonucleoprotein D3 polypeptide	2403.18
NFYC ^a nuclear transcription factor Y, gamma	714.2
HNRPDL heterogeneous nuclear ribonucleoprotein D-like	933.48
PAIP1 polyadenylate binding protein-interacting protein 1	6194.88
DEK DEK oncogene (DNA binding)	3655.95
TAF1B TATA box binding protein (TBP)-associated factor, RNA polymerase II, B, 63 kD	13,192.28
SRP14 signal recognition particle 14 kD (homologous Alu RNA-binding protein)	3807.8
HIF1A hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	6556.6
SFRS7 splicing factor, arginine/serine-rich 7 (35 kD)	935.42
TARDBP TAR DNA binding protein	5490.13
TAF2C1 TATA box binding protein (TBP)-associated factor, RNA polymerase II, C1, 130 kD	6558.18
DDXBP1 DEAD/H (Asp–Glu–Ala–Asp/His) box binding protein 1	3439.45
POU4F1 ^a POU domain, class 4, transcription factor 1	1973.9
RBM4 RNA binding motif protein 4	4231.23
NR1H4 ^a nuclear receptor family 1, group H, member 4	770.25
NFE2L2 nuclear factor (erythroid-derived 2)-like 2	908.1
GABPB2 ^a GA-binding protein transcription factor, beta subunit 1 (53 kD)	1500.38
SFRS10 splicing factor, arginine/serine-rich (transformer 2 <i>Drosophila</i> homologue) 10	8348.13
ICSBP1 interferon consensus sequence binding protein 1	5153.38
RAD50 RAD50 (<i>S. cerevisiae</i>) homologue	818.95
RFC3 replication factor C (activator 1) 3 (38 kD)	887.2
SRP54 signal recognition particle 54 kD	563.43
TAF2D TATA box binding protein (TBP)-associated factor, RNA polymerase II, D, 100 kD	21,199.6
MNPEP methionine aminopeptidase; eIF-2-associated p67	765.93
ILF2 interleukin enhancer binding factor 2, 45 kD	502.4
TCEB1 transcription elongation factor B (SIII), polypeptide 1 (15 kD, elongin C)	6280.08
RNF11 ring finger protein 11	745.35
HNRPD heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA-binding protein 1, 37 kD)	1541.48
GTF2H1 general transcription factor IIIH, polypeptide 1 (62 kD subunit)	2631.98
SF3B1 ^a splicing factor 3b, subunit 1	8245.45
SF3A1 ^a splicing factor 3a, subunit 1, 60 kD	3018.07
SRP19 signal recognition particle 19 kD	2469.73
PHF2 PHD finger protein 2	1625.95
EIF4G2 ^a eukaryotic translation initiation factor 4 gamma, 2	2969.7
EIF4G1 ^a eukaryotic translation initiation factor 4 gamma, 1	370.2
SFRS5 splicing factor, arginine/serine-rich 5	981

Table 1 contd

SNAPC1 ^a small nuclear RNA activating complex, polypeptide 1	711.58
SNAPC5 ^a small nuclear RNA activating complex, polypeptide 5	623.68
<i>Heterogeneous</i>	
BPGM 2,3-bisphosphoglycerate mutase	2322.15
LDHA ^a lactate dehydrogenase A	1090.13
LDHC ^a lactate dehydrogenase C	841.67
LCP host cell factor homologue	6515.63
CPD ^a carboxypeptidase D	1966.5
TPD52L2 tumour protein D52-like 2	1182.32
OAZ ornithine decarboxylase antizyme	2992.1
MEST mesoderm specific transcript (mouse) homologue	1296.9
MKLN1 muskelin 1, intracellular mediator containing kelch motifs	1746.97
TNRC3 trinucleotide repeat containing 3	2397.1
MRG15 MORF-related gene 15	22,931.38
LOC51582 antizyme inhibitor	551.53
TBCE tubulin-specific chaperone e	2290.53
TC21 oncogene TC21	2593.13
BAZ1A ^a bromodomain adjacent to zinc finger domain, 1A	1151.7
ADAR adenosine deaminase, RNA specific	1848.63
DAZAP2 DAZ associated protein	850.07
SET SET translocation (myeloid leukaemia-associated)	5825.52
PFDN14 prefoldin 4	417.8
TAX1BP1 Tax 1 (human T-cell leukaemia virus type I) binding protein 1	52,278.77
MAGOH mago-nashi (<i>Drosophila</i>) homologue, proliferation-associated	7313.35
DLD dihydrolipoamide dehydrogenase	1080.7
AMD1 S-adenosylmethionine decarboxylase 1	1726.35
HPRT1 hypoxanthine phosphoribosyl transferase 1 (Lesch–Nyhan syndrome)	17,135.18
SPS2 selenophosphate synthetase 2	1598.25
SEP15 15 kDa selenoprotein	953.83
ITSN2 intersectin 2	602.5
CCT8 ^a chaperonin containing TCP1, subunit 8	9620.88
CCT4 ^a chaperonin containing TCP1, subunit 4	6441.45
AMPD3 adenosine monophosphate deaminase (isoform E)	6551.95
HSD17B4 ^a hydroxysteroid (17-beta) dehydrogenase 4	2674.25
HSPE1 heat shock 10 kD protein 1 (chaperonin 10)	841
YWHAQ tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	37,587.6
TMP21 transmembrane trafficking protein	569.15
LOC51230 hepatocellular carcinoma-associated antigen 58	742.52
SUCLA2 succinate-CoA ligase, ADP-forming, beta subunit	571.38
RPA1 replication protein A1 (70 kD)	17,461.22
PRPSAP1 phosphoribosyl pyrophosphate synthetase-associated protein 1	980.25
B4GALT3 ^a UDP-Gal: beta GlcNAc beta 1,4-galactosyltransferase, polypeptide 3	842.45
TACC1 ^a transforming, acidic coiled-coil containing protein 1	11,599.88
TACC2 ^a transforming, acidic coiled-coil containing protein 2	672.57
ENPP2 ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	13,523.35
DOC-1R deleted in oral cancer-related 1	2591.55
BETA3GNT ^a beta-1,3-N-acetylglucosaminyltransferase	2716.18
GA17 dendritic cell protein	570.25
SEC24B ^a SEC24 (<i>S. cerevisiae</i>) related gene family, member B	4094.65
CA12 ^a carbonic anhydrase XII	489.83
HSPD1 heat shock 60 kD protein 1 (chaperonin)	598.13
TBCA tubulin-specific chaperone a	22,413.15
NDUFB2 ^a NADH dehydrogenase (ubiquinone) 1 beta complex, 2	5002.92
P4HA1 procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I	977.5
AUH AU RNA-binding protein/enoyl coenzyme A hydratase	4097.42
SAT spermidine/spermine N1-acetyltransferase	2175.5
YWHAH tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	736.88

Table 1 contd

P85SPR PAK interacting exchange factor beta	1698.7
FNTA farnesyltransferase CAAX box, alpha	3166.07
HABP2 hyaluronan-binding protein 2	1207.95
UGDH UDP-glucose dehydrogenase	2403.95
D1S155E NRAS-related gene	6330.02
PPAA ^a peptidylprolyl isomerase (cyclophilin A) A	4304.48
MYCBP c-myc binding protein	1087.85
ODC1 ornithine decarboxylase 1	13,702.25
RAD21 RAD21 (<i>S. pombe</i>) homologue	987.47
PRSC1 protease, cysteine, 1 (legumain)	1403.47
TXNL thioredoxin-like, 32 kD	1781.52
PTMA prothymosin, alpha (gene sequence 28)	1132.15
HERC2 hect domain and RLD2	2825.13
GTPBP ^a GTP binding protein	1185.6
TTC3 ^a tetratricopeptide repeat domain 3	4935.97
MTX2 ^a metaxin 2	334.55
YME1L1 YME1 (<i>S. cerevisiae</i>)-like 1	395.25
BRD4 bromodomain-containing 4	606.95
HYP2 ^a huntingtin interacting protein 2	2732.25
DSCR2 Down syndrome critical region gene 2	5771.55
UAP1 UDP-N-acetylglucosamine pyrophosphorylase 1	1112.68
NDUFA10 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10 (42 kD)	9487.58
IDE insulin-degrading enzyme	
NDUFS5 ^a NADH dehydrogenase (ubiquinone) Fe-S protein 5 (15 kD) NADH-coenzyme Q reductase	499.7
NDUFAB1 ^a NADH dehydrogenase (ubiquinone) 1, alphabeta subcomplex, 1 (8 kD, SDAP)	2121.45
NDUFB8 ^a NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 8 (19 kD, ASH1)	30,888.18
NDUFB1 ^a NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 1 (7 kD, MNLL)	1862.43
NDUFB5 ^a NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5 (16 kD, SGDHD)	1805.57
SC5DL sterol-C5-desaturase (fungal ERG3, delta-5 desaturase)-like	658.67
LOC51205 LPAP for lysophosphatidic acid phosphatase	569.88
KNSL2 ^a kinesin-like 2	5766.8
KNSL5 ^a kinesin-like 5	1293.22
NT5B 5'-nucleotidase (purine), cytosolic type B	603.6
CSDA cold shock domain protein	319.38
MAT2A methionine adenosyl transferase II, alpha	5101.9
SC4MOL sterol-C4-methyl oxidase-like	836.97
LOC51142 16.7 kD protein	847.08
CNIL cornichon-like	2680.7
PDHB ^a pyruvate dehydrogenase (lipoamide) beta	2240.15
LOC55892 myoneurin	2777.3
CDS1 CDP-diacylglycerol synthase (phosphatidate cytidyl transferase) 1	2922.65

^aDenotes closely related isoform.**Table 2.** Characteristics of single oocytes and pooled oocytes preparations.

Sample type	Total genes expressed	Exclusively expressed genes	Genes in common
Single oocytes	1467	106	1361
Multiple oocytes	1823	462	

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