

**GENES THAT ARE UP- OR DOWN-REGULATED
DURING DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS**

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BACKGROUND

A promising development in the field of regenerative medicine has been the isolation and propagation of human stem cells from the early embryo. These cells have two very special properties: First, unlike other normal mammalian cell types, they can be propagated in culture almost indefinitely, providing a virtually unlimited supply. Second, they can be used to generate a variety of tissue types of interest as a source of replacement cells and tissues for use in therapy.

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Thomson et al. (Science 282:114, 1998; U.S. Patent 6,200,806) were the first to successfully isolate and propagate embryonic stem cells from human blastocysts. Gearhart and coworkers derived human embryonic germ cell lines from fetal gonadal tissue (Shamblott et al., Proc. Natl. Acad. Sci. USA 95:13726, 1998; U.S. Patent 6,090,622).

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International Patent Publication WO 99/20741 (Geron Corp.) describes methods and materials for the growth of primate-derived primordial stem cells. International Patent Publication WO 01/51616 (Geron Corp.) provides techniques for growth and differentiation of human pluripotent stem cells. An article by Xu et al. (Nature Biotechnology 19:971, 2001) describes feeder-free growth of undifferentiated human embryonic stem cells. Lebkowski et al. (Cancer J. 7 Suppl. 2:S83, 2001) discuss the culture, differentiation, and genetic modification of human embryonic stem cell for regenerative medicine applications. These publications report exemplary culture methods for propagating human embryonic stem cells in an undifferentiated state, and their use in preparing cells for human therapy.

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Markers for identifying undifferentiated pluripotent stem cells include SSEA-4, Tra-1-60, and Tra-1-81 (Thomson et al. and Gearhart et al., supra). They also express human telomerase reverse transcriptase, and the POU transcription factor Oct 3/4 (WO 01/51616; Amit et al., Dev. Biol. 227:271, 2000; Xu et al., supra).

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Loring et al. (Restor. Neurol. Neurosci. 18:81, 2001) review gene expression profiles of embryonic stem cells and ES-derived neurons. Pesce et al. (Bioessays 20:722, 1998) comment on the potential role of transcription factor Oct-4 in the totipotent germ-line cycle of mice. Gajovic et al. (Exp. Cell Res. 242:138, 1998) report that genes expressed after retinoic acid-mediated differentiation of embryoid bodies are likely to be expressed during embryo development. Zur Nieden et al. (Toxicol. in Vitro 15:455, 2001) propose certain molecular markers for embryonic stem cells. Henderson et al. (Stem Cells 20:329, 2002) report that pre-implantation human embryos and ES cells have comparable expression of SSEAs. Tanaka et al. (Genome Res. 12:1921, 2002) profile gene expression in mouse ES cells to identify candidate genes associated with pluripotency and lineage specificity. Draper et al. (J. Anat. 299:249, 2002) review change of surface antigens of human embryonic stem cells upon differentiation in culture.

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Kelly et al. (Mol Reprod. Dev. 56:113, 2000) report DNA microarray analyses of genes regulated during the differentiation of embryonic stem cells. Woltjen et al. (Nucl. Acids Res. 28:E41, 2000) report retro-recombination screening of a mouse embryonic stem cell genomic library. Monk et al. (Oncogene

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20:8085, 2001) list human embryonic genes re-expressed in cancer cells. Tanaka et al. (Genome Res. 12:1921, 2002) discuss gene expression profiling of embryo-derived stem cells, and candidate genes putatively associated with pluripotency and lineage specificity. Monk et al. report developmental genes identified by differential display (Reprod. Fertil. Dev. 13:51, 2001). Natale et al. (Reprod. 122:687, 2001) characterize bovine blastocyst gene expression patterns by differential display RT-PCR.

Fan et al. (Dev. Biol. 210:481, 1999) propose that forced expression of the homeobox-containing gene *Pem* blocks differentiation of embryonic stem cells. Abdel-Rahman et al. (Hum. Reprod. 10:2787, 1995) report the effect of expressing transcription regulating genes in human preimplantation embryos. Jackson et al. (J. Biol. Chem. 277:38683, 2002) describe the cloning and characterization of *Ehox*, a homeobox gene that reportedly plays a role in ES cell differentiation.

The following disclosure provides new markers and marker combinations that are effective means to identify, characterize, qualify, and control differentiation of pluripotent cells.

SUMMARY OF THE INVENTION

This invention identifies a number of genes that are up- or down-regulated during the course of differentiation of early-stage pluripotent stem cells obtained from primates, exemplified by human embryonic stem cells. As a consequence, the genes are differentially expressed in undifferentiated versus differentiated cells. This property confers special benefit on these genes for identification, characterization, culturing, differentiation, and manipulation of stem cells and their progeny, and other cells that express the same markers.

One aspect of this invention is a system for assessing a culture of undifferentiated primate pluripotent stem (pPS) cells or their progeny, in which expression of one or more of the identified markers listed in the disclosure is detected or measured. The level of expression can be measured in isolation or compared with any suitable standard, such as undifferentiated pPS cells maintained under specified conditions, progeny at a certain stage of differentiation, or stable end-stage differentiated cells, such as may be obtained from the ATCC. Depending on whether the marker(s) are up- or down-regulated during differentiation, presence of the markers is correlated with the presence or proportion of undifferentiated or differentiated cells in the population.

An exemplary (non-limiting) combination suitable for qualifying cultures of pPS cells are markers of the undifferentiated phenotype selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein, hTERT and/Oct 3/4 (POU domain, class 5 transcription factor), in various combinations. Other cell markers can be measured in conjunction, including any of the newly described differentiation markers listed in this disclosure, or traditional pPS cell markers like SSEA-4 and Tra-1-60. In addition, markers of various differentiated cell phenotypes can be assayed as a measure of contaminating cells. Early stage cell types include stromal cells (marked by CD44 and Vimentin), fibroblasts, mesenchymal cells, and embryoid body cells. The markers can be detected or quantified at the mRNA level by PCR amplification, at the protein or enzyme product level by antibody assay, or by any suitable technique.

The marker system of this invention can be used for quantifying the proportion of undifferentiated pPS cells or differentiated cells in the culture; for assessing the ability of a culture system or component

thereof (such as a soluble factor, culture medium, or feeder cell) to maintain pPS cells in an undifferentiated state; for assessing the ability of a culture system or component thereof to cause differentiation of pPS cells into a culture of lineage-restricted precursor cells or terminally differentiated cells; or for any other worthwhile purpose. This invention includes kits and the use of specific reagents in order to measure the expression of the markers whenever appropriate.

This invention also provides a system assessing the growth characteristics of a cell population by detecting or measuring expression of one or more of the differentially expressed marker genes identified in this disclosure. This can be applied not only to various types of pPS cells and progenitor cells in various stages of differentiation, but also to clinical samples from a disease condition associated with abnormal cell growth. Renewed expression of markers of a relatively undifferentiated phenotype may be diagnostic of disease conditions such as cancer, and can serve as a means by which to target therapeutic agents to the disease site.

The marker system can also be used to regulate gene expression. Transcriptional control elements for the markers will cause an operatively linked encoding region to be expressed preferentially in undifferentiated or differentiated cells. For example, the encoding sequence can be a reporter gene (such as a gene that causes the cells to emit fluorescence), a positive selection marker (such as a drug resistance gene), or a negative selection marker. Vector constructs comprising recombinant elements linked in this fashion can be used to positively select or deplete undifferentiated, differentiated, or cancerous cells from a mixed population or in vivo, depending on the nature of the effector gene and whether transcription is up- or down-regulated during differentiation. They can also be used to monitor culture conditions of pPS cells, differentiation conditions, or for drug screening.

The marker system of this invention can also be used to sort differentiated cells from less differentiated cells. The marker can be used directly for cell separation by adsorption using an antibody or lectin, or by fluorescence activated cell sorting. Alternatively, these separation techniques can be effected using a transcription promoter from the marker gene in a promoter-reporter construct.

The marker system of this invention can be used to map differentiation pathways or influence differentiation. Markers suited for this purpose may act as transcription regulators, or encode products that enhance cell interaction in some fashion. pPS cells or their differentiated progeny are genetically altered to increase expression of one or more of the identified genes using a transgene, or to decrease expression, for example, using an antisense or siRNA construct. Alternatively, gene products involved in cell interaction or signaling can be added directly to the culture medium. The effect of this can be to help maintain the transfected cell in the undifferentiated state, promote differentiation in general, or direct differentiation down a particular pathway.

Another aspect of the invention are methods for identifying these and other genes that are up- or down-regulated upon differentiation of any cell type. The methods involve comparing expression libraries obtained from the cells before and after differentiation, by sequencing transcripts in each of the libraries, and identifying genes that have statistically significant differences in the relative number of transcripts (as a percentage of transcripts in each library) at a confidence level of 67%, 95%, or 98%. The method can be enhanced by creating assemblies in which different sequences are counted for the same transcript if they are known to correspond to a single transcript according to previously compiled data.

Amongst the differentially expressed markers identified in this disclosure are 39 nucleotide sequences which are not present in their entirety in the UniGene database. These are listed in this

disclosure as SEQ. ID NOs:1 to 39. This invention includes novel nucleic acids consisting of or containing any of these sequences or the complementary sequences, and novel fragments thereof. This invention also includes novel polypeptides encoded in these sequences (made either by expressing the nucleic acid or by peptide synthesis), antibodies specific for the polypeptides (made by conventional techniques or through a commercial service), and use of these nucleic acids, peptides, and antibodies for any industrial application.

Also embodied in this invention are culture conditions and other cell manipulations identified using the marker system of this invention that are suitable for maintaining or proliferating pPS cells without allowing differentiation, or causing them to differentiate in a certain fashion. Culture conditions tested and validated according to this invention are illustrated in the example section.

Other embodiments of the invention will be apparent from the description that follows.

DRAWINGS

Figure 1 shows the profile of genes preferentially expressed in undifferentiated pluripotent stem cells, upon preliminary differentiation of the cells by culturing in retinoic acid or DMSO. Level of gene expression at the mRNA level was measured by real-time PCR assay. Any of the genes showing substantial down-regulation upon differentiation can be used to characterize the undifferentiated cell population, and culture methods suitable for maintaining them in an undifferentiated state.

Figure 2 shows the level of expression of five genes in hES cells, compared with fully differentiated cells. This five-marker panel provides robust qualification of the undifferentiated phenotype.

Figure 3 show results of an experiment in which hES cells of the H1 line were maintained for multiple passages in different media. Medium conditioned with feeder cells provides factors effective to allow hES cells to proliferate in culture without differentiating. However, culturing in unconditioned medium leads to decreased percentage of cells expressing CD9, and the classic hES cell marker SSEA-4.

Figure 4 illustrates the sensitivity of hTERT, Oct 3/4, Cripto, GRP receptor, and podocalyxin-like protein (measured by real-time PCR) as a means of determining the degree of differentiation of the cells. After multiple passages in unconditioned medium, all five markers show expression that has been downregulated by 10 to 10⁴-fold.

Figure 5 shows results of an experiment in which the hES cell line H1 was grown on different feeder cell lines: mEF = mouse embryonic fibroblasts; hMSC = human mesenchymal stem cells; UtSMC = uterine smooth muscle cells; WI-38 = human lung fibroblasts. As monitored using Cripto, the hMSC is suitable for use as feeder cells to promote hES cell proliferation without differentiation.

Figure 6 shows results of an experiment in which different media were tested for their ability to promote growth of hES cells without proliferation. The test media were not preconditioned, but supplemented with 8-40 ng/mL bFGF, with or without stem cell factor, Flt3 ligand, or LIF. Effective combinations of factors (Conditions 4 to 8) were identified by following the undifferentiated phenotype using the markers of this invention. Alterations in expression profiles were temporary and reversible, showing that the cells are still undifferentiated.

Figure 7 shows analysis of the undifferentiated hES cell markers SSEA-4, TRA 1-60 and Oct-4 by antibody staining and flow cytometry. Oct-4 is detected by permeabilizing the cells before staining.

Figure 8 shows the results of the immunocytochemical analysis for stromal cell markers CD44, STRO-1 and Vimentin, which label cells in the hES cell culture that have undergone differentiation.

Figure 9 shows the relative gene expression levels for cell populations in which undifferentiated hES cells were mixed with BJ fibroblasts in increasing amounts.

DETAILED DESCRIPTION

The propensity of pluripotent stem cells to differentiate spontaneously has made it challenging for investigators to work with these cells. Consistent cultures of undifferentiated stem cells are required to compare results obtained from multiple experiments performed within or between laboratories. Unfortunately, morphological characterization is subjective and especially difficult for cultures that often contain 10-20% differentiated cells. Nevertheless, having a set of standardized criteria will be important in qualifying these cells for use in clinical therapy.

The marker system identified in this disclosure provides the basis for establishing these standards. 148,453 different transcripts were amplified and sequenced from undifferentiated human embryonic stem cells, and three types of progeny. As a result of this sequencing effort, 532 genes were identified having substantially higher EST counts in undifferentiated cells, and 142 genes were identified having substantially higher EST counts after differentiation. Other differentially expressed genes were identified by microarray analysis of undifferentiated cells, compared with cells at the beginning of the differentiation process.

The system provided by this invention can be used not only to qualify populations of undifferentiated cells, but in other powerful ways of maintaining and manipulating cells described later in this disclosure. Culture systems have been identified and protocols have been developed to expand cultures of undifferentiated cells and produce commercially viable quantities of cells for use in research, drug screening, and regenerative medicine.

Definitions

"Pluripotent Stem cells" (pPS cells) are pluripotent cells that have the characteristic of being capable under appropriate conditions of producing progeny of several different cell types that are derivatives of all of the three germinal layers (endoderm, mesoderm, and ectoderm), according to a standard art-accepted test, such as the ability to form a teratoma in 8-12 week old SCID mice. The term includes both established lines of stem cells of various kinds, and cells obtained from primary tissue that are pluripotent in the manner described. For the purposes of this disclosure, the pPS cells are not embryonal carcinoma (EC) cells, and are not derived from a malignant source. It is desirable (but not always necessary) that the cells be euploid. Exemplary pPS cells are obtained from embryonic or fetal tissue at any time after fertilization.

"Human Embryonic Stem cells" (hES cells) are pluripotent stem cells derived from a human embryo in the blastocyst stage, or human pluripotent cells produced by artificial means (such as by

nuclear transfer) that have equivalent characteristics. Exemplary derivation procedures and features are provided in a later section.

hES cell cultures are described as "undifferentiated" when a substantial proportion (at least 20%, and possibly over 50% or 80%) of stem cells and their derivatives in the population display morphological characteristics of undifferentiated cells, distinguishing them from differentiated cells of embryo or adult origin. It is understood that colonies of undifferentiated cells within the population will often be surrounded by neighboring cells that are differentiated. It is also understood that the proportion of cells displaying the undifferentiated phenotype will fluctuate as the cells proliferate and are passaged from one culture to another. Cells are recognized as proliferating in an undifferentiated state when they go through at least 4 passages and/or 8 population doublings while retaining at least about 50%, or the same proportion of cells bearing characteristic markers or morphological characteristics of undifferentiated cells.

A "differentiated cell" is a cell that has progressed down a developmental pathway, and includes lineage-committed progenitor cells and terminally differentiated cells.

"Feeder cells" or "feeders" are terms used to describe cells of one type that are co-cultured with cells of another type, to provide an environment in which the cells of the second type can grow. hES cell populations are said to be "essentially free" of feeder cells if the cells have been grown through at least one round after splitting in which fresh feeder cells are not added to support the growth of pPS cells.

The term "embryoid bodies" refers to aggregates of differentiated and undifferentiated cells that appear when pPS cells overgrow in monolayer cultures, or are maintained in suspension cultures. Embryoid bodies are a mixture of different cell types, typically from several germ layers, distinguishable by morphological criteria and cell markers detectable by immunocytochemistry.

A cell "marker" is any phenotypic feature of a cell that can be used to characterize it or discriminate it from other cell types. A marker of this invention may be a protein (including secreted, cell surface, or internal proteins; either synthesized or taken up by the cell); a nucleic acid (such as an mRNA, or enzymatically active nucleic acid molecule) or a polysaccharide. Included are determinants of any such cell components that are detectable by antibody, lectin, probe or nucleic acid amplification reaction that are specific for the cell type of interest. The markers can also be identified by a biochemical or enzyme assay that depend on the function of the gene product. Associated with each marker is the gene that encodes the transcript, and the events that lead to marker expression.

A marker is said to be "preferentially expressed" in an undifferentiated or differentiated cell population, if it is expressed at a level that is at least 10 times higher (in terms of total gene product measured in an antibody or PCR assay) or 10 times more frequently (in terms of positive cells in the population). Markers that are expressed 100, 1,000, or 10,000 times higher or more frequently are increasingly more preferred.

The terms "polynucleotide" and "nucleic acid" refer to a polymeric form of nucleotides of any length. Included are genes and gene fragments, mRNA, cDNA, plasmids, viral and non-viral vectors and particles, nucleic acid probes, amplification primers, and their chemical equivalents. As used in this disclosure, the term polynucleotide refers interchangeably to double- and single-stranded molecules. Unless otherwise specified, any embodiment of the invention that is a polynucleotide encompasses both a double-stranded form, and each of the two complementary single-stranded forms known or predicted to make up the double-stranded form.

A cell is said to be "genetically altered" or "transfected" when a polynucleotide has been transferred into the cell by any suitable means of artificial manipulation, or where the cell is a progeny of the originally altered cell that has inherited the polynucleotide.

A "control element" or "control sequence" is a nucleotide sequence involved in an interaction of molecules that contributes to the functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. "Operatively linked" refers to an operative relationship between genetic elements, in which the function of one element influences the function of another element. For example, an expressible encoding sequence may be operatively linked to a promoter that drives gene transcription.

The term "antibody" as used in this disclosure refers to both polyclonal and monoclonal antibody. The ambit of the term deliberately encompasses not only intact immunoglobulin molecules, but also such fragments and derivatives of immunoglobulin molecules that retain a desired binding specificity.

General Techniques

Methods in molecular genetics and genetic engineering are described generally in the current editions of Molecular Cloning: A Laboratory Manual, (Sambrook et al.); Oligonucleotide Synthesis (M.J. Gait, ed.); Animal Cell Culture (R.I. Freshney, ed.); Gene Transfer Vectors for Mammalian Cells (Miller & Calos, eds.); Current Protocols in Molecular Biology and Short Protocols in Molecular Biology, 3rd Edition (F.M. Ausubel et al., eds.); and Recombinant DNA Methodology (R. Wu ed., Academic Press). Antibody production is described in Basic Methods in Antibody Production and Characterization (Howard & Bethell eds., CRC Press, 2000).

A survey of relevant techniques is provided in such standard texts as DNA Sequencing (A.E. Barron, John Wiley, 2002), and DNA Microarrays and Gene Expression (P. Baldi et al., Cambridge U. Press, 2002). For a description of the molecular biology of cancer, the reader is referred to Principles of Molecular Oncology (M.H. Bronchud et al. eds., Humana Press, 2000); The Biological Basis of Cancer (R.G. McKinnel et al. eds., Cambridge University Press, 1998); and Molecular Genetics of Cancer (J.K. Cowell ed., Bios Scientific Publishers, 1999).

Sources of Stem Cells

This invention is based on observations made with established lines of hES cells. The markers are suitable for identifying, characterizing, and manipulating related types of undifferentiated pluripotent cells. They are also suitable for use with pluripotent cells obtained from primary embryonic tissue, without first establishing an undifferentiated cell line. It is contemplated that the markers described in this application will in general be useful for other types of pluripotent cells, including embryonic germ cells (U.S. Patents 6,090,622 and 6,251,671), and ES and EG cells from other mammalian species, such as non-human primates.

Embryonic Stem Cells

Embryonic stem cells can be isolated from blastocysts of members of primate species (U.S. Patent 5,843,780; Thomson et al., Proc. Natl. Acad. Sci. USA 92:7844, 1995). Human embryonic stem (hES) cells can be prepared from human blastocyst cells using the techniques described by Thomson et

al. (U.S. Patent 6,200,806; Science 282:1145, 1998; Curr. Top. Dev. Biol. 38:133 ff., 1998) and Reubinoff et al, Nature Biotech. 18:399, 2000. Equivalent cell types to hES cells include their pluripotent derivatives, such as primitive ectoderm-like (EPL) cells, outlined in WO 01/51610 (Bresagen).

hES cells can be obtained from human preimplantation embryos. Alternatively, in vitro fertilized (IVF) embryos can be used, or one-cell human embryos can be expanded to the blastocyst stage (Bongso et al., Hum Reprod 4: 706, 1989). Embryos are cultured to the blastocyst stage in G1.2 and G2.2 medium (Gardner et al., Fertil. Steril. 69:84, 1998). The zona pellucida is removed from developed blastocysts by brief exposure to pronase (Sigma). The inner cell masses are isolated by immunosurgery, in which blastocysts are exposed to a 1:50 dilution of rabbit anti-human spleen cell antiserum for 30 min, then washed for 5 min three times in DMEM, and exposed to a 1:5 dilution of Guinea pig complement (Gibco) for 3 min (Solter et al., Proc. Natl. Acad. Sci. USA 72:5099, 1975). After two further washes in DMEM, lysed trophectoderm cells are removed from the intact inner cell mass (ICM) by gentle pipetting, and the ICM plated on mEF feeder layers.

After 9 to 15 days, inner cell mass derived outgrowths are dissociated into clumps, either by exposure to calcium and magnesium-free phosphate-buffered saline (PBS) with 1 mM EDTA, by exposure to dispase or trypsin, or by mechanical dissociation with a micropipette; and then replated on mEF in fresh medium. Growing colonies having undifferentiated morphology are individually selected by micropipette, mechanically dissociated into clumps, and replated. ES-like morphology is characterized as compact colonies with apparently high nucleus to cytoplasm ratio and prominent nucleoli. Resulting ES cells are then routinely split every 1-2 weeks by brief trypsinization, exposure to Dulbecco's PBS (containing 2 mM EDTA), exposure to type IV collagenase (~200 U/mL; Gibco) or by selection of individual colonies by micropipette. Clump sizes of about 50 to 100 cells are optimal.

Propagation of pPS Cells in an Undifferentiated State

pPS cells can be propagated continuously in culture, using culture conditions that promote proliferation without promoting differentiation. Exemplary serum-containing ES medium is made with 80% DMEM (such as Knock-Out DMEM, Gibco), 20% of either defined fetal bovine serum (FBS, Hyclone) or serum replacement (US 20020076747 A1, Life Technologies Inc.), 1% non-essential amino acids, 1 mM L-glutamine, and 0.1 mM β -mercaptoethanol. Just before use, human bFGF is added to 4 ng/mL (WO 99/20741, Geron Corp.).

Traditionally, ES cells are cultured on a layer of feeder cells, typically fibroblasts derived from embryonic or fetal tissue. Embryos are harvested from a CF1 mouse at 13 days of pregnancy, transferred to 2 mL trypsin/EDTA, finely minced, and incubated 5 min at 37°C. 10% FBS is added, debris is allowed to settle, and the cells are propagated in 90% DMEM, 10% FBS, and 2 mM glutamine. To prepare a feeder cell layer, cells are irradiated to inhibit proliferation but permit synthesis of factors that support ES cells (~4000 rads γ -irradiation). Culture plates are coated with 0.5% gelatin overnight, plated with 375,000 irradiated mEFs per well, and used 5 h to 4 days after plating. The medium is replaced with fresh hES medium just before seeding pPS cells.

Scientists at Geron have discovered that pPS cells can be maintained in an undifferentiated state even without feeder cells. The environment for feeder-free cultures includes a suitable culture substrate, particularly an extracellular matrix such as Matrigel® or laminin. The pPS cells are plated at >15,000

cells cm^{-2} (optimally 90,000 cm^{-2} to 170,000 cm^{-2}). Typically, enzymatic digestion is halted before cells become completely dispersed (say, ~5 min with collagenase IV). Clumps of ~10 to 2,000 cells are then plated directly onto the substrate without further dispersal. Alternatively, the cells can be harvested without enzymes before the plate reaches confluence by incubating ~5 min in a solution of 0.5 mM EDTA in PBS. After washing from the culture vessel, the cells are plated into a new culture without further dispersal. In a further illustration, confluent human embryonic stem cells cultured in the absence of feeders are removed from the plates by incubating with a solution of 0.05% (wt/vol) trypsin (Gibco) and 0.053 mM EDTA for 5-15 min at 37°C. The remaining cells in the plate are removed and the cells are triturated into a suspension comprising single cells and small clusters, and then plated at densities of 50,000-200,000 cells cm^{-2} to promote survival and limit differentiation.

Feeder-free cultures are supported by a nutrient medium containing factors that support proliferation of the cells without differentiation. Such factors may be introduced into the medium by culturing the medium with cells secreting such factors, such as irradiated (~4,000 rad) primary mouse embryonic fibroblasts, telomerized mouse fibroblasts, or fibroblast-like cells derived from pPS cells. Medium can be conditioned by plating the feeders at a density of $\sim 5\text{-}6 \times 10^4 \text{ cm}^{-2}$ in a serum free medium such as KO DMEM supplemented with 20% serum replacement and 4 ng/mL bFGF. Medium that has been conditioned for 1-2 days is supplemented with further bFGF, and used to support pPS cell culture for 1-2 days. Alternatively or in addition, other factors can be added that help support proliferation without differentiation, such as ligands for the FGF-2 or FGF-4 receptor, ligands for c-kit (such as stem cell factor), ligands for receptors associated with gp130, insulin, transferrin, lipids, cholesterol, nucleosides, pyruvate, and a reducing agent such as β -mercaptoethanol. Aspects of the feeder-free culture method are further discussed in International Patent Publications WO 99/20741, WO 01/51616; Xu et al., Nat. Biotechnol. 19:971, 2001; and PCT application PCT/US02/28200. Exemplary culture conditions tested and validated using the marker system of this invention are provided below in Example 6.

Under the microscope, ES cells appear with high nuclear/cytoplasmic ratios, prominent nucleoli, and compact colony formation with poorly discernable cell junctions. Conventional markers for hES cells are stage-specific embryonic antigen (SSEA) 3 and 4, and markers detectable using antibodies Tra-1-60 and Tra-1-81 (Thomson et al., Science 282:1145, 1998). Differentiation of pPS cells in vitro results in the loss of SSEA-4, Tra-1-60, and Tra-1-81 expression, and increased expression of SSEA-1.

Markers of undifferentiated pPS cells and their differentiated progeny

The tables and description provided later in this disclosure provide markers that distinguish undifferentiated pPS cells from their differentiated progeny.

Expression libraries were made from ES cells (WO 01/51616), embryoid bodies (WO 01/51616), and cells differentiated towards the hepatocyte (WO 01/81549) or neural cell (WO 01/88104) lineage. mRNA was reverse transcribed and amplified, producing expressed sequence tags (ESTs) occurring in frequency proportional to the level of expression in the cell type being analyzed. The ESTs were subjected to automatic sequencing, and counted according to the corresponding unique (non-redundant) transcript. A total of 148,453 non-redundant transcripts were represented in each of the 4 libraries. Genes were then identified as having a differential expression pattern if the number of EST counts of the transcript was statistically different between the libraries being compared.

In a parallel set of experiments, mRNA from each of the cell types was analyzed for binding to a broad-specificity EST-based microarray, performed according to the method described in WO 01/51616. Genes were identified as having a differential expression pattern if they showed a comparatively different signal on the microarray.

Significant expression differences determined by EST sequencing, microarray analysis, or other observations were confirmed by real-time PCR analysis. The mRNA was amplified by PCR using specific forward and reverse primers designed from the GenBank sequence, and the amplification product was detected using labeled sequence-specific probes. The number of amplification cycles required to reach a threshold amount was then compared between different libraries.

Distinguishing markers fall into several categories. Those of particular interest include the following:

- Markers characteristically expressed at a higher level in undifferentiated pPS cells than any of the differentiated cells, indicating down-regulation during differentiation. The gene products may be involved in maintaining the undifferentiated phenotype.
- Markers characteristically expressed at a higher level in the three differentiated cell types than in the undifferentiated cells, indicating up-regulation during differentiation. The gene products may be involved in the general differentiation process.
- Markers characteristically expressed at a higher level in one of the differentiated cell types. The encoded genes may be involved in differentiation down restricted lineages.

Markers can also be classified according to the function of the gene product or its location in the cell. Where not already indicated, protein gene products can be predicted by referencing public information according to the GenBank accession number, or by translating the open reading frame after the translation start signal through the genetic code. Features of the markers listed can be determined by the descriptors given in the tables below, or by using the accession number or sequence data to reference public information. Marker groups of particular interest include the following:

- Secreted proteins — of interest, for example, because they can be detected by immunoassay of the culture supernatant, and may transmit signals to neighboring cells. Secreted proteins typically have an N-terminal signal peptides, and may have glycosylation sites.
- Surface membrane proteins — of interest, for example, because they can be used for cell-surface labeling and affinity separation, or because they act as receptors for signal transduction. They may have glycosylation sites and a membrane spanning region. A Markov model for predicting transmembrane protein topology is described by Krogh et al., J. Mol Biol. 305:567, 2001.
- Enzymes with relevant function. For example, enzymes involved in protein synthesis and cleavage or in apoptosis may influence differentiation. Glycosyltransferases decorate the cell membrane with distinguishing carbohydrate epitopes that may play a role in cellular adhesion or localization.
- Transcription regulatory factors — of interest for their potential to influence differentiation, as explained later in this disclosure. These factors sometimes have zinc fingers or other identifiable topological features involved in the binding or metabolism of nucleic acids.

Through the course of this work, the key signaling pathways Wnt, Sonic hedgehog (Shh), and Notch emerged as regulators of growth of pPS cells. Interestingly, these pathways have also been shown to play a role in the growth of tumor cells of various kinds, and in embryonic development of lower species.

Now that genes have been identified that are up-regulated or down-regulated upon differentiation, a number of commercial applications of these markers will be apparent to the skilled reader. The sections that follow provide non-limiting illustrations of how some of these embodiments can be implemented.

Use of cell markers to characterize pPS cells and their differentiated progeny

The markers provided in this disclosure can be used as a means to identify both undifferentiated and differentiated cells — either a population as a whole, or as individual cells within a population. This can be used to evaluate the expansion or maintenance of pre-existing cell populations, or to characterize the pluripotent nature (or lineage commitment) of newly obtained populations.

Expression of single markers in a test cell will provide evidence of undifferentiated or differentiated phenotype, according to the expression pattern listed later in this disclosure. A plurality of markers (such as any 2, 3, 4, 5, 6, 8, 10, 12, 15, or 20 markers from Tables 2-3 or 5-9) will provide a more detailed assessment of the characteristics of the cell. Expression of genes that are down-regulated and/or lack of expression of genes that are up-regulated upon differentiation correlates with a differentiated phenotype. Expression of genes that are up-regulated and/or lack of expression of genes that are down-regulated upon differentiation correlates with an undifferentiated phenotype. The markers newly identified in this disclosure may be analyzed together (with or without markers that were previously known) in any combination effective for characterizing the cell status or phenotype.

Exemplary combinations of markers are provided elsewhere in this disclosure. For determining the undifferentiated cell phenotype, combinations of markers like Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), and human telomerase reverse transcriptase (hTERT) are effective, either alone, or in combination with cell surface markers like SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81, or intracellular markers like Oct 3/4. For determining differentiated cells, any marker can be used that is characteristic of contaminating cells that may be present. Depending on culture conditions, early stage non-specific hES cell differentiation generates cells having characteristics of stromal cells, fibroblasts, mesenchymal cells, embryoid body cells, and other cell types. Alternatively, a combination of markers characteristic of several types of cells can be used, as long as they are preferentially expressed in differentiated cells.

Tissue-specific markers can be detected using any suitable immunological technique — such as flow cytometry for cell-surface markers, or immunocytochemistry (for example, of fixed cells or tissue sections) for intracellular or cell-surface markers. Expression of a cell-surface antigen is defined as positive if a significantly detectable amount of antibody will bind to the antigen in a standard immunocytochemistry or flow cytometry assay, optionally after fixation of the cells, and optionally using a labeled secondary antibody or other conjugate to amplify labeling.

The expression of tissue-specific gene products can also be detected at the mRNA level by Northern blot analysis, dot-blot hybridization analysis, or by reverse transcriptase initiated polymerase chain reaction (RT-PCR) using sequence-specific primers in standard amplification methods. See U.S.

Patent No. 5,843,780 for further details. Sequence data for particular markers listed in this disclosure can be obtained from public databases such as GenBank.

These and other suitable assay systems are described in standard reference texts, such as the following: PCR Cloning Protocols, 2nd Ed (James & Chen eds., Humana Press, 2002); Rapid Cycle Real-Time PCR: Methods and Applications (C. Wittwer et al. eds., Springer-Verlag NY, 2002); Immunoassays: A Practical Approach (James Gosling ed., Oxford Univ Press, 2000); Cytometric Analysis of Cell Phenotype and Function (McCarthy et al. eds., Cambridge Univ Press, 2001). Reagents for conducting these assays, such as nucleotide probes or primers, or specific antibody, can be packaged in kit form, optionally with instructions for the use of the reagents in the characterization or monitoring of pPS cells, or their differentiated progeny.

Use of cell markers for clinical diagnosis

Stem cells regulate their own replenishment and serve as a source of cells that can differentiate into defined cell lineages. Cancer cells also have the ability to self-renew, but lack of regulation results in uncontrolled cellular proliferation. Three key signaling pathways, Wnt, Sonic hedgehog (Shh), and Notch, are known growth regulators of tumor cells. The genomics data provided in this disclosure indicate that all three of these pathways are active in hES cells.

It is a hypothesis of this invention that many of the markers discovered to be more highly expressed in undifferentiated pPS cells can also be up-regulated upon dedifferentiation of cells upon malignant transformation. Accordingly, this disclosure provides a system for evaluating clinical conditions associated with abnormal cell growth, such as hyperplasia or cancers of various kinds. Markers meeting the desired criteria include those contained in Tables 2, 5, 7 and 9.

Expression of each marker of interest is determined at the mRNA or protein level using a suitable assay system such as those described earlier; and then the expression is correlated with the clinical condition that the patient is suspected of having. As before, combinations of multiple markers may be more effective in doing the assessment. Presence of a particular marker may also provide a means by which a toxic agent or other therapeutic drug may be targeted to the disease site.

In a similar fashion, the markers of this invention can be used to evaluate a human or non-human subject who has been treated with a cell population or tissue generated by differentiating pPS cells. A histological sample taken at or near the site of administration, or a site to which the cells would be expected to migrate, could be harvested at a time subsequent to treatment, and then assayed to assess whether any of the administered cells had reverted to the undifferentiated phenotype. Reagents for conducting diagnostic tests, such as nucleotide probes or primers, or specific antibody, can be packaged in kit form, optionally with instructions for the use of the reagents in the determination of a disease condition.

Use of cell markers to assess and manipulate culture conditions

The markers and marker combinations of this invention provide a system for monitoring undifferentiated pPS cells and their differentiated progeny in culture. This system can be used as a quality control, to compare the characteristics of undifferentiated pPS cells between different passages or

different batches. It can also be used to assess a change in culture conditions, to determine the effect of the change on the undifferentiated cell phenotype.

Where the object is to produce undifferentiated cells, a decrease in the level of expression of an undifferentiated marker because of the alteration by 3-, 10-, 25-, 100- and 1000-fold is progressively less preferred. Corresponding increases in marker expression may be more beneficial. Moderate decreases in marker expression may be quite acceptable within certain boundaries, if the cells retain their ability to form progeny of all three germ layers is retained, and/or the level of the undifferentiated marker is relatively restored when culture conditions are returned to normal.

In this manner, the markers of this invention can be used to evaluate different feeder cells, extracellular matrixes, base media, additives to the media, culture vessels, or other features of the culture as illustrated in WO 99/20741 and PCT application PCT/US02/28200. Illustrations of this technique are provided below in Example 6 (Figures 3 to 6).

In a similar fashion, the markers of this invention can also be used to monitor and optimize conditions for differentiating cells. Improved differentiation procedures will lead to higher or more rapid expression of markers for the differentiated phenotype, and/or lower or more rapid decrease in expression of markers for the undifferentiated phenotype.

Use of cell markers to regulate gene expression

Differential expression of the markers listed in this disclosure indicates that each marker is controlled by a transcriptional regulatory element (such as a promoter) that is tissue specific, causing higher levels of expression in undifferentiated cells compared with differentiated cells, or vice versa. When the corresponding transcriptional regulatory element is combined with a heterologous encoding region to drive expression of the encoding region, then the expression pattern in different cell types will mimic that of the marker gene.

Minimum promoter sequences of many of the genes listed in this disclosure are known and further described elsewhere. Where a promoter has not been fully characterized, specific transcription can usually be driven by taking the 500 base pairs immediately upstream of the translation start signal for the marker in the corresponding genomic clone.

To express a heterologous encoding region according to this embodiment of the invention, a recombinant vector is constructed in which the specific promoter of interest is operatively linked to the encoding region in such a manner that it drives transcription of the encoding region upon transfection into a suitable host cell. Suitable vector systems for transient expression include those based on adenovirus and certain types of plasmids. Vectors for long-term expression include those based on plasmid lipofection or electroporation, episomal vectors, retrovirus, and lentivirus.

One application of tissue-specific promoters is expression of a reporter gene. Suitable reporters include fluorescence markers such as green fluorescent protein, luciferase, or enzymatic markers such as alkaline phosphatase and β -galactosidase. Other reporters such as a blood group glycosyltransferase (WO 02/074935), or Invitrogen's pDisplay™, create a cell surface epitope that can be counterstained with labeled specific antibody or lectin. pPS cells labeled with reporters can be used to follow the differentiation process directly, the presence or absence of the reporter correlating with the undifferentiated or differentiated phenotype, depending on the specificity of the promoter. This in turn can

be used to follow or optimize culture conditions for undifferentiated pPS cells, or differentiation protocols. Alternatively, cells containing promoter-reporter constructs can be used for drug screening, in which a test compound is combined with the cell, and expression or suppression of the promoter is correlated with an effect attributable to the compound.

5 Another application of tissue-specific promoters is expression of a positive or negative drug selection marker. Antibiotic resistance genes such as neomycin phosphotransferase, expressed under control of a tissue-specific promoter, can be used to positively select for undifferentiated or differentiated cells in a medium containing the corresponding drug (geneticin), by choosing a promoter with the appropriate specificity. Toxin genes, genes that mediate apoptosis, or genes that convert a prodrug into
10 a toxic compound (such as thymidine kinase) can be used to negatively select against contaminating undifferentiated or differentiated cells in a population of the opposite phenotype (WO 02/42445; GB 2374076).

Promoters specific for the undifferentiated cell phenotype can also be used as a means for targeting cancer cells — using the promoter to drive expression of a gene that is toxic to the cell
15 (WO 98/14593, WO 02/42468), or to drive a replication gene in a viral vector (WO 00/46355). For example, an adenoviral vector in which the GRPR promoter (AY032865) drives the E1a gene should specifically lyse cancer cells in the manner described in Majumdar et al., Gene Ther. 8:568, 2001. Multiple promoters for the undifferentiated phenotype can be linked for improved cancer specificity (USSN 10/206,447).

20 Other useful applications of tissue-specific promoters of this invention will come readily to the mind of the skilled reader.

Use of markers for cell separation or purification

Differentially expressed markers provided in this disclosure are also a means by which mixed cell
25 populations can be separated into populations that are more homogeneous. This can be accomplished directly by selecting a marker of the undifferentiated or differentiated phenotype, which is itself expressed on the cell surface, or otherwise causes expression of a unique cell-surface epitope. The epitope is then used as a handle by which the marked cells can be physically separated from the unmarked cells. For example, marked cells can be aggregated or adsorbed to a solid support using an antibody or lectin that
30 is specific for the epitope. Alternatively, the marker can be used to attach a fluorescently labeled antibody or lectin, and then the cell suspension can be subject to fluorescence-activated cell sorting.

An alternative approach is to take a tissue-specific promoter chosen based on its expression pattern (as described in the last section), and use it to drive transcription of a gene suitable for separating the cells. In this way, the marker from which the promoter is chosen need not itself be a cell surface
35 protein. For example, the promoter can drive expression of a fluorescent gene, such as GFP, and then cells having the marked phenotype can be separated by FACS. In another example, the promoter drives expression of a heterologous gene that causes expression of a cell-surface epitope. The epitope is then used for adsorption-based separation, or to attach a fluorescent label, as already described.

Use of cell markers to influence differentiation

In another embodiment of this invention, the differentially expressed genes of this invention are caused to increase or decrease their expression level, in order to either inhibit or promote the differentiation process. Suitable genes are those that are believed in the normal case of ontogeny to be active in maintaining the undifferentiated state, active in the general process of differentiation, or active in differentiation into particular cell lineages. Markers of interest for this application are the following:

- Transcription factors and other elements that directly affect transcription of other genes, such as Forkhead box O1A (FOXO1A); Zic family member 3 (ZIC3); Hypothetical protein FLJ20582; Forkhead box H1 (FOXH1); Zinc finger protein, Hsal2; KRAB-zinc finger protein SZF1-1; Zinc finger protein of cerebellum ZIC2; and Coup transcription factor 2 (COUP-TF2). Other candidates include those marked in Tables 5 and 6 with the symbol "⊗", and other factors with zinc fingers or nucleic acid binding activity.
- Genes that influence cell interaction, such as those that encode adhesion molecules, and enzymes that make substrates for adhesion molecules
- Genes encoding soluble factors that transmit signals within or between cells, and specific receptors that recognize them and are involved in signal transduction.

One way of manipulating gene expression is to induce a transient or stable genetic alteration in the cells using a suitable vector, such as those already listed. Scientists at Geron Corp. have determined that the following constitutive promoters are effective in undifferentiated hES cells: for transient expression CMV, SV40, EF1 α , UbC, and PGK; for stable expression, SV40, EF1 α , UbC, MND and PGK. Expressing a gene associated with the undifferentiated phenotype may assist the cells to stay undifferentiated in the absence of some of the elements usually required in the culture environment. Expressing a gene associated with the differentiated phenotype may promote early differentiation, and/or initiate a cascade of events beneficial for obtaining a desired cell population. Maintaining or causing expression of a gene of either type early in the differentiation process may in some instances help guide differentiation down a particular pathway.

Another way of manipulating gene expression is to alter transcription from the endogenous gene. One means of accomplishing this is to introduce factors that specifically influence transcription through the endogenous promoter. Another means suitable for down-regulating expression at the protein level is to genetically alter the cells with a nucleic acid that removes the mRNA or otherwise inhibits translation (for example, a hybridizing antisense molecule, ribozyme, or small interfering RNA). Dominant-negative mutants of the target factor can reduce the functional effect of the gene product. Targeting a particular factor associated with the undifferentiated phenotype in this fashion can be used to promote differentiation. In some instances, this can lead to de-repression of genes associated with a particular cell type.

Where the gene product is a soluble protein or peptide that influences cell interaction or signal transduction (for example, cytokines like osteopontin and Cripto), then it may be possible to affect differentiation simply by adding the product to the cells — in either recombinant or synthetic form, or purified from natural sources. Products that maintain the undifferentiated phenotype can then be withdrawn from the culture medium to initiate differentiation; and products that promote differentiation can be withdrawn once the process is complete.

Since differentiation is a multi-step process, changing the level of gene product on a permanent basis may cause multiple effects. In some instances, it may be advantageous to affect gene expression in a temporary fashion at each sequential step in the pathway, in case the same factor plays different effects at different steps of differentiation. For example, function of transcription factors can be evaluated by changing expression of individual genes, or by invoking a high throughput analysis, using cDNAs obtained from a suitable library such as exemplified in Example 1. Cells that undergo an alteration of interest can be cloned and pulled from multi-well plates, and the responsible gene identified by PCR amplification.

The effect of up- or down-regulating expression of a particular gene can be determined by evaluating the cell for morphological characteristics, and the expression of other characteristic markers. Besides the markers listed later in this disclosure, the reader may want to follow the effect on particular cell types, using markers for later-stage or terminally differentiated cells. Tissue-specific markers suitable for this purpose are listed in WO 01/81549 (hepatocytes), WO 01/88104 (neural cells), PCT/US02/20998 (osteoblasts and mesenchymal cells), PCT/US02/22245 (cardiomyocytes), PCT/US02/39091 (hematopoietic cells), PCT/US02/39089 (islet cells), and PCT/US02/39090 (chondrocytes). Such markers can be analyzed by PCR amplification, fluorescence labeling, or immunocytochemistry, as already described. Promoter-reporter constructs based on the same markers can facilitate analysis when expression is being altered in a high throughput protocol.

*The examples that follow are provided for further illustration,
and are not meant to limit the claimed invention.*

EXAMPLES

Example 1: An EST database of undifferentiated hES cells and their differentiated progeny

cDNA libraries were prepared from human embryonic stem (hES) cells cultured in undifferentiated form. cDNA libraries were also prepared from progeny, subject to non-specific differentiation as embryoid bodies (EBs), or taken through the preliminary stages of established differentiation protocols for neurons (preNEU) or hepatocytes (preHEP).

The hES cell lines H1, H7, and H9 were maintained under feeder-free conditions. Cultures were passaged every 5-days by incubation in 1 mg/mL collagenase IV for 5-10 min at 37°C, dissociated and seeded in clumps at 2.5 to 10×10^5 cells/well onto Matrigel™-coated six well plates in conditioned medium supplemented with 8 mg/mL bFGF. cDNA libraries were made after culturing for 5 days after the last passage.

EBs were prepared as follows. Confluent plates of undifferentiated hES cells were treated briefly with collagenase IV, and scraped to obtain small clusters of cells. Cell clusters were resuspended in 4 mL/well differentiation medium (KO DMEM containing 20% fetal bovine serum in place of 20% SR, and not preconditioned) on low adhesion 6-well plates (Costar). After 4 days in suspension, the contents of each well was transferred to individual wells pre-coated with gelatin. Each well was re-fed with 3 mL fresh differentiation medium every two days after replating. Cells were used for the preparation of cytoplasmic RNA on the eighth day after plating.

PreHEP cells were prepared based on the hepatocyte differentiation protocol described in WO 01/81549. Confluent wells of undifferentiated cells were prepared, and medium was changed to KO DMEM plus 20% SR + 1% DMSO. The medium was changed every 24 h, and cells were used for preparation of cytoplasmic RNA on day 5 of DMSO treatment.

PreNEU cells were prepared based on the neural differentiation protocol described in WO 01/88104. hES cells of the H7 line (p29) were used to generate EBs as described above except that 10 μ M all-trans RA was included in the differentiation medium. After 4 days in suspension, EBs were transferred to culture plate precoated with poly-L-lysine and laminin. After plating, the medium was changed to EPFI medium. Cells were used for the preparation of cytoplasmic RNA after 3 days of growth in EPFI.

Partial 5' end sequences (an expressed sequence tag, or EST) were determined by conventional means for independent clones derived from each cDNA library. Overlapping ESTs were assembled into conjoined sequences.

TABLE 1: Non-redundant EST sequences

Library	Number of ESTs
hESC	37,081
EB	37,555
preHEP	35,611
preNEU	38,206
Total	148,453

All of the stem cell lines used for preparation of the expression libraries were originally isolated and initially propagated on mouse feeder cells. Accordingly, the libraries were analyzed to determine whether they were contaminated with murine retroviruses that had shed from the feeder cells and subsequently infected the stem cells. Three complete viral genomes were used in a BLAST search: Moloney murine leukemia virus, Friend murine leukemia virus, and murine type C retrovirus. No matches with a high score were found against any of the ESTs.

The sequences were then compared to the Unigene database of human genes. ESTs that were at least 98% identical, over a stretch of at least 150 nucleotides each, to a common reference sequence in Unigene, were assumed to be transcribed from the same gene, and placed into a common assembly. The complete set of 148,453 ESTs collapsed to a non-redundant set of 32,764 assemblies.

Example 2: Selection of marker genes specific for undifferentiated and differentiated cells

Candidate markers were selected from a database based on the imputed level of gene expression. The frequency of ESTs for any particular gene correlates with the abundance of that mRNA in the cells used to generate the cDNA library. Thus, a comparison of frequencies of ESTs among the libraries indicates the relative abundance of the associated mRNA in the different cell types.

Candidate molecular markers were selected from the expressed gene (EST) database from their greater abundance in undifferentiated hES cells, relative to differentiated hES cells. Genes were identified as having a differential expression pattern (being up- or down-regulated) during the differentiation process, if the count of ESTs sequenced in the undifferentiated cells was substantially different from the sum of ESTs in the three differentiated libraries.

Oct 3/4 (a POU domain-containing transcription factor) and telomerase reverse transcriptase (hTERT) are known to be expressed preferentially in undifferentiated hES cells (WO 01/51616). Other genes suitable for characterizing or manipulating the undifferentiated phenotype are those that are down-regulated upon differentiation with a significance of $p \leq 0.05$, as determined by the Fisher Exact Test (explained below). 193 genes were found to have 4-fold more ESTs in hES cells, relative to each of the three cell types. 532 genes were found that were 2-fold greater hES cells, with a confidence of over 95% as determined by the Fisher Exact Test, relative to the sum of ESTs of the three cell types (minimum of 4 ESTs in hES cells). The following markers are of particular interest:

TABLE 2: EST Frequency of Genes that are Down-regulated upon Differentiation of hES cells

Geron ID	GenBank ID	Name	EST counts			
			ES	EB	preHEP	preNEU
GA_10902	NM_024504	Pr domain containing 14 (PRDM14)	12	1	0	0
GA_11893	NM_032805	Hypothetical protein FLJ14549	25	0	0	0
GA_12318	NM_032447	Fibrillin3	6	0	0	0
GA_1322	NM_000142	Fibroblast growth factor receptor 3 precursor (FGFR-3)	9	1	5	1
GA_34679	NM_002015	Forkhead box o1a (FOXO1a)	4	0	1	1
GA_1470	NM_003740	potassium channel, subfamily K, member 5 (KCNK5), mRNA	4	0	0	1
GA_1674	NM_002701	Octamer-Binding Transcription Factor 3a (OCT-3A) (OCT-4)	24	1	2	0
GA_2024	NM_003212	Teratocarcinoma-derived growth factor 1 (CRIPTO)	20	1	0	0
GA_2149	NM_003413	Zic family member 3 (ZIC3)	7	0	1	0
GA_2334	NM_000216	Kallmann syndrome 1 sequence (KAL1)	5	0	1	0
GA_23552	NM_152742	hypothetical protein DKFZp547M109 (DKFZp547M109), mRNA	6	0	1	2
GA_2356	NM_002851	Protein tyrosine phosphatase, receptor-type, z polypeptide 1 (PTPRZ1),	10	0	0	0
GA_2357	NM_001670	Armadillo repeat protein deleted in velo-cardio-facial syndrome (ARVCF)	6	0	0	0
GA_23578	BM454360	AGENCOURT_6402318 NIH_MGC_85 Homo sapiens cDNA clone IMAGE:5497491	6	0	0	0

TABLE 2: EST Frequency of Genes that are Down-regulated upon Differentiation of hES cells

Geron ID	GenBank ID	Name	EST counts			
			ES	EB	preHEP	preNEU
5', mRNA sequence						
GA_2367	NM_003923	Forkhead box H1 (FOXH1)	5	0	0	0
GA_2436	NM_004329	Bone morphogenetic protein receptor, type Ia (BMPRI1A) (ALK-3)	7	3	1	1
GA_2442	NM_004335	Bone marrow stromal antigen 2 (BST-2)	13	0	2	3
GA_2945	NM_005232	Ephrin type-a receptor 1 (EPHA1)	5	1	1	1
GA_2962	NM_005314	Gastrin-releasing peptide receptor (GRP-R)	4	0	0	0
GA_2988	NM_005397	Podocalyxin-like (PODXL)	59	23	5	8
GA_3337	NM_006159	NELL2 (nel-like protein 2)	5	3	2	0
GA_3559	NM_005629	Solute carrier family 6, member 8 (SLC6A8)	5	1	0	1
GA_3898	NM_006892	DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B)	49	2	3	1
GA_5391	NM_002968	Sal-like 1 (SALL1),	7	1	1	0
GA_33680	NM_016089	Krab-zinc finger protein SZF1-1	15	0	1	0
GA_36977	NM_020927	KIAA1576 protein	9	2	1	0
GA_8723	NM_152333	Homo sapiens chromosome 14 open reading frame 69 (C14orf69), mRNA	14	1	1	3
GA_9167	AF308602	Notch 1 (N1)	6	2	1	0
GA_9183	NM_007129	Homo sapiens Zic family member 2 (odd-paired homolog, Drosophila) (ZIC2), mRNA	8	1	1	0
GA_35037	NM_004426	Homo sapiens polyhomeotic-like 1 (Drosophila) (PHC1), mRNA	34	9	5	4

Only one EST for hTERT was identified in undifferentiated hES cells and none were detected from the differentiated cells, which was not statistically significant. Thus, potentially useful markers that are expressed at low levels could have been omitted in this analysis, which required a minimum of four ESTs. It would be possible to identify such genes by using other techniques described elsewhere in this disclosure.

Three genes were observed from EST frequency queries that were of particular interest as potentially useful markers of hES cells. They were Teratocarcinoma-derived growth factor (Cripto), Podocalyxin-like (PODXL), and gastrin-releasing peptide receptor (GRPR). These genes were not only

more abundant in undifferentiated cells, relative to differentiated hES cells, but also encoded for proteins expressed on the surface of cells. Surface markers have the added advantage that they could be easily detected with immunological reagents. ESTs for Cripto and GRPR were quite restricted to hES cells, with one or zero ESTs, respectively, scored in any of the differentiated cells. PODXL ESTs were detected in all 4-cell types, but substantially fewer (2.5X -12X) in differentiated cells. All three markers retained a detectable level of expression in differentiated cultures of hES cells. There may be a low level of expression of these markers in differentiated cells, or the expression detected may be due to a small proportion of undifferentiated cells in the population. GABA(A) receptor, Lefty B, Osteopontin, Thy-1 co-transcribed, and Solute carrier 21 are other significant markers of the undifferentiated phenotype.

By similar reasoning, genes that show a higher frequency of ESTs in differentiated cells can be used as specific markers for differentiation. ESTs that are 2-fold more abundant in the sum of all three differentiated cell types (EBs, preHEP and preNEU cells) and with a p-value ≤ 0.05 as determined by the Fisher Exact Test, compared with undifferentiated hES cells are candidate markers for differentiation down multiple pathways. ESTs that are relatively abundant in only one of the differentiated cell types are candidate markers for tissue-specific differentiation. The following markers are of particular interest:

TABLE 3: EST Frequency of Genes that are Upregulated upon Differentiation

Geron ID	GenBank ID	Name	EST counts			
			ES	EB	preHEP	preNEU
GA_35463	NM_024298	Homo sapiens leukocyte receptor cluster (LRC) member 4 (LENG4), mRNA	0	4	9	8
GA_10492	NM_006903	Inorganic pyrophosphatase (PPASE)	0	5	5	6
GA_38563	NM_021005	Homo sapiens nuclear receptor subfamily 2, group F, member 2 (NR2F2), mRNA	0	9	8	9
GA_38570	NM_001844	Collagen, type II, alpha 1 (COL2A1), transcript variant 1		15	31	5
GA_1476	NM_002276	Keratin type I cytoskeletal 19 (cytokeratin 19)	1	26	14	38
GA_34776	NM_002273	Keratin type II cytoskeletal 8 (cytokeratin 8) (CK 8)	9	71	144	156
GA_1735	NM_002806	Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 6 (PSMC6), mRNA	1	7	7	8
GA_1843	NM_000982	60s ribosomal protein l21	1	7	48	42
GA_35369	NM_003374	Voltage-dependent anion-selective channel (VDAC-1)	1	5	6	10
GA_23117	NM_004772	P311 protein [Homo sapiens]	1	5	7	6
GA_2597	NM_138610	Homo sapiens H2A histone family, member Y (H2AFY), transcript variant 3, mRNA	1	5	5	14
GA_3283	NM_004484	Homo sapiens glypican 3 (GPC3), mRNA	1	6	7	12

TABLE 3: EST Frequency of Genes that are Upregulated upon Differentiation

Geron ID	GenBank ID	Name	EST counts			
			ES	EB	preHEP	preNEU
GA_3530	NM_002539	Homo sapiens ornithine decarboxylase 1 (ODC1), mRNA	1	10	8	9
GA_4145	NM_002480	Protein phosphatase 1, regulatory(inhibitor) subunit 12A (PPP1R12A)	1	6	6	6
GA_5992	NM_014899	Homo sapiens Rho-related BTB domain containing 3 (RHOBTB3), mRNA	0	10	7	13
GA_6136	NM_016368	Homo sapiens myo-inositol 1-phosphate synthase A1 (ISYNA1), mRNA	1	7	5	16
GA_6165	NM_015853	Orf (LOC51035)	1	5	9	5
GA_6219	NM_016139	16.7Kd protein (LOC51142),	1	5	13	14
GA_723	NM_005801	Homo sapiens putative translation initiation factor (SUI1), mRNA	1	14	15	19
GA_9196	NM_000404	Homo sapiens galactosidase, beta 1 (GLB1), transcript variant 179423, mRNA	0	6	10	7
GA_9649	NM_014604	Tax interaction protein 1 (TIP-1)	0	8	5	5

The relative expression levels were calculated as follows:

$$\begin{aligned}
 es &= \frac{(\# \text{ ESTs of the gene in hES cells} \div \text{total unique genes in hES cells})}{(\# \text{ ESTs of the gene in differentiated cells} \div \text{total unique genes in differentiated cells})} \\
 &= \frac{(\# \text{ ESTs for the gene in hES cells} \div 37,081)}{(\# \text{ ESTs for the gene in differentiated cells} \div 111,372)}
 \end{aligned}$$

5

The es value is substantially >1 for genes marking the undifferentiated phenotype, and <1 for genes indicating differentiation.

The Fisher Exact Test was used to determine whether changes were statistically significant.

10 S. Siegel & N.J. Castellan. *Nonparametric Statistics for the Behavioral Sciences* (2nd ed., McGraw-Hill NJ, 1988). This is a standard test that can be used for 2×2 tables, and is conservative in declaring significance if the data are sparse. For analysis of EST sequences, the tables were of the following form:

TABLE 4: Fisher Exact Test for Statistical Analysis of Differential Expression

	Gene X	All Other Genes	Total
Pool A	a = number of sequences in Pool A assigned to Gene X	A = number of sequences in Pool A NOT assigned to Gene X	N = a + A total number of sequences in Pool A
Pool B	b = number of sequences in Pool B assigned to Gene X	B = number of sequences in Pool B NOT assigned to Gene X	M = b + B total number of sequences in Pool B
Total	c = a + b	C = A + B	N + M = c + C

where Pool A contains the sequences derived from the undifferentiated hES cells and Pool B contains the sequences from the other three cell types (EB, preHep, preNeu). N is equal to the number of sequences derived from the undifferentiated hES cells (37,081) and M is equal to the sum of all ESTs from the three differentiated cell types (111,372). For any given pair of pool sizes (N, M) and gene counts (c and C), the probability p of the table being generated by chance is calculated where:

$$p = [N! M! c! C!] / [(N + M)! a! b! A! B!]$$

and where 0! by default is set to 1. The null hypothesis of a gene being equally represented in two pools is rejected when probability $p \leq 0.05$, where 0.05 is the level of statistical certainty. Thus, genes with $p \leq 0.05$ are considered to be differentially represented.

The following markers were identified as changing their expression levels significantly upon differentiation. The markers identified with the symbol "⊗" may play a role in the regulation of gene transcription.

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_10021	NM_018124	hypothetical protein FLJ10520 (FLJ10520)		1	0	3	10	es 4.51	p=0.02
GA_10053	NM_033427	cortactin binding protein 2 (CORTBP2)	4	0	0	0	4	es >4	p=0.00
GA_10057	AB051540	KIAA1753 protein sequence	4	1	1	0	6	es 6.01	p=0.04
GA_10082	NM_030645	KIAA1720 protein (KIAA1720)	6	0	1	0	7	es 18.02	p=0.00
GA_10153	NM_015039	chromosome 1 open reading frame 15 (C1orf15), transcript variant 1	4	1	1	0	6	es 6.01	p=0.04
GA_102	NM_015043	KIAA0676 protein (KIAA0676)	6	4	0	1	11	es 3.60	p=0.03
GA_10252	NM_003376	vascular endothelial growth factor (VEGF)	5	2	0	2	9	es 3.75	p=0.05
GA_10258	AK091948	cDNA FLJ34629 fis, clone KIDNE2015515, highly similar to NADP-dependent leukotriene b4 12-hydroxydehydrogenase (EC 1.1.1.-) sequence	4	0	0	0	4	es >4	p=0.00
GA_10308	NM_024046	hypothetical protein MGC8407 (MGC8407)	4	0	0	0	4	es >4	p=0.00
GA_10327	NM_024077	SECIS binding protein 2 (SBP2)	9	2	3	2	16	es 3.86	p=0.01
GA_10334	NM_024090	long-chain fatty-acyl elongase (LCE)	5	0	0	2	7	es 7.51	p=0.01
GA_10513	NM_033209	Thy-1 co-transcribed (LOC94105)	7	2	2	1	12	es 4.20	p=0.01
GA_10528	NM_030622	cytochrome P450, subfamily IIS, polypeptide 1 (CYP2S1)	6	0	1	0	7	es 18.02	p=0.00
GA_1053	NM_001618	ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase) (ADPRT)	25	13	14	9	61	es 2.09	p=0.01
GA_10531	NM_015271	tripartite motif-containing 2 (TRIM2)	6	2	0	2	10	es 4.51	p=0.02

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_10603	NM_025215	pseudouridylate synthase 1 (PUS1)	5	0	2	2	9	es 3.75	p=0.05
GA_10641	NM_025108	hypothetical protein FLJ13909 (FLJ13909)	6	0	0	1	7	es 18.02	p=0.00
GA_10649	NM_025082	hypothetical protein FLJ13111 (FLJ13111)	8	3	0	0	11	es 8.01	p=0.00
GA_1067	NM_020977	ankyrin 2, neuronal (ANK2), transcript variant 2	4	0	0	0	4	es >4	p=0.00
GA_10696	NM_024888	hypothetical protein FLJ11535 (FLJ11535)	5	2	0	0	7	es 7.51	p=0.01
GA_10713	NM_024844	pericentrin 1 (PCNT1)	8	1	1	0	10	es 12.01	p=0.00
GA_1076	NM_001659	ADP-ribosylation factor 3 (ARF3)	19	8	5	4	36	es 3.36	p=0.00
GA_10831	NM_024619	hypothetical protein FLJ12171 (FLJ12171)	4	0	1	1	6	es 6.01	p=0.04
GA_1085	NM_000048	argininosuccinate lyase (ASL)	6	2	0	0	8	es 9.01	p=0.00
GA_10902	NM_024504	PR domain containing 14 (PRDM14)	12	1	0	0	13	es 36.04	p=0.00
GA_10905	NM_022362	MMS19-like (MET18 homolog, S. cerevisiae) (MMS19L)	10	5	4	1	20	es 3.00	p=0.02
GA_10935	NM_032569	cytokine-like nuclear factor n-pac (N-PAC)	8	3	1	1	13	es 4.81	p=0.01
GA_11047	NM_004728	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21 (DDX21)	18	9	3	5	35	es 3.18	p=0.00
GA_11103	NM_138347	hypothetical protein BC005868 (LOC90233)	4	0	2	0	6	es 6.01	p=0.04
GA_1119	NM_001217	carbonic anhydrase XI (CA11)	5	1	2	1	9	es 3.75	p=0.05
GA_11368	NM_032147	hypothetical protein DKFZp434D0127 (DKFZP434D0127)	7	1	0	0	8	es 21.02	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_11398	NM_015471	DKFZP566O1646 protein (DC8)	5	1	1	0	7	es 7.51 p=0.01
GA_11528	NM_021633	kelch-like protein C3IP1 (C3IP1)	5	1	0	1	7	es 7.51 p=0.01
GA_11532	NM_024900	PHD protein Jade-1 (Jade-1)	⊗	6	1	0	2	es 6.01 p=0.01
GA_11552	NM_024086	hypothetical protein MGC3329 (MGC3329)	6	3	0	1	10	es 4.51 p=0.02
GA_11577	AB058780	KIAA1877 protein sequence	4	2	0	0	6	es 6.01 p=0.04
GA_1160	NM_052988	cyclin-dependent kinase (CDC2-like) 10 (CDK10), transcript variant 3	4	0	1	1	6	es 6.01 p=0.04
GA_11600	NM_002883	Ran GTPase activating protein 1 (RANGAP1)	12	7	3	5	27	es 2.40 p=0.03
GA_11656	NM_018425	phosphatidylinositol 4-kinase type II (PI4KII)	5	1	1	2	9	es 3.75 p=0.05
GA_11773	NM_025109	hypothetical protein FLJ22865 (FLJ22865)	6	0	0	0	6	es >4 p=0.00
GA_11790	NM_013432	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-like 2 (NFKBIL2)	⊗	5	2	0	0	es 7.51 p=0.01
GA_11868	NM_032844	hypothetical protein FLJ14813 (FLJ14813)	6	2	1	1	10	es 4.51 p=0.02
GA_11893	NM_032805	hypothetical protein FLJ14549 (FLJ14549)	25	0	0	0	25	es >4 p=0.00
GA_11964	NM_032620	mitochondrial GTP binding protein (GTPBG3)	5	1	1	2	9	es 3.75 p=0.05
GA_11971	NM_138575	hypothetical protein MGC5352 (MGC5352)	4	1	1	0	6	es 6.01 p=0.04
GA_12025	NM_020465	NDRG family member 4 (NDRG4)	4	1	0	0	5	es 12.01 p=0.02
GA_12064			4	1	0	0	5	es 12.01 p=0.02

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_1212	NM_001313	collapsin response mediator protein 1 (CRMP1)	7	1	1	2	11	es 5.26	p=0.01
GA_12167	NM_138357	hypothetical protein BC010682 (LOC90550)	4	0	0	0	4	es >4	p=0.00
GA_1217	NM_001316	CSE1 chromosome segregation 1-like (yeast) (CSE1L)	23	7	5	2	37	es 4.93	p=0.00
GA_12173	NM_021912	gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3), transcript variant 2	4	0	0	0	4	es >4	p=0.00
GA_12253	NM_032420	protocadherin 1 (cadherin-like 1) (PCDH1), transcript variant 2	5	0	0	2	7	es 7.51	p=0.01
GA_12279	NM_033019	PCTAIRE protein kinase 1 (PCTK1), transcript variant 3	11	7	2	4	24	es 2.54	p=0.03
GA_12318	NM_032447	fibrillin3 (KIAA1776)	6	0	0	0	6	es >4	p=0.00
GA_1236	NM_003611	oral-facial-digital syndrome 1 (OFD1)	4	0	1	0	5	es 12.01	p=0.02
GA_12367	NM_033317	hypothetical gene ZD52F10 (ZD52F10)	8	1	4	4	17	es 2.67	p=0.05
GA_12386	AB002336	KIAA0338 sequence	4	1	0	0	5	es 12.01	p=0.02
GA_12440	NM_032383	Hermansky-Pudlak syndrome 3 (HPS3)	7	1	0	0	8	es 21.02	p=0.00
GA_12522	NM_052860	kruppel-like zinc finger protein (ZNF300)	6	2	2	1	11	es 3.60	p=0.03
GA_1260	NM_000791	dihydrofolate reductase (DHFR)	15	4	2	4	25	es 4.51	p=0.00
GA_12630	NM_015356	scribble (SCRIB)	12	4	0	2	18	es 6.01	p=0.00
GA_12635	NM_002913	replication factor C (activator 1) 1, 145kDa (RFC1)	8	0	1	0	9	es 24.03	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_12640	NM_004741	nucleolar and coiled-body phosphoprotein 1 (NOLC1)	16	9	7	6	38	es 2.18	p=0.02
GA_1265	NM_001387	dihydropyrimidinase-like 3 (DPYSL3)	39	13	3	14	69	es 3.90	p=0.00
GA_12672	D86976	similar to C.elegans protein (Z37093) sequence	5	2	0	1	8	es 5.01	p=0.03
GA_12767	NM_015360	KIAA0052 protein (KIAA0052)	8	2	2	1	13	es 4.81	p=0.01
GA_12899	BC039246	clone IMAGE:5278517	5	2	1	1	9	es 3.75	p=0.05
GA_12900	NM_003302	thyroid hormone receptor interactor 6 (TRIP6)	12	3	3	4	22	es 3.60	p=0.00
GA_12949	BC033781	PAX transcription activation domain interacting protein 1 like sequence	4	0	0	1	5	es 12.01	p=0.02
GA_12954	NM_003972	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae) (BTAF1)	7	3	2	0	12	es 4.20	p=0.01
GA_1322	NM_000142	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism) (FGFR3), transcript variant 1	9	1	5	1	16	es 3.86	p=0.01
GA_1378	NM_000178	glutathione synthetase (GSS)	4	0	1	1	6	es 6.01	p=0.04
GA_1386	NM_001517	general transcription factor IIH, polypeptide 4 (52kD subunit) (GTF2H4)	8	1	2	2	13	es 4.81	p=0.01
GA_1470	NM_003740	potassium channel, subfamily K, member 5 (KCNK5)	4	0	0	1	5	es 12.01	p=0.02
GA_1523	NM_002442	musashi homolog 1 (Drosophila) (MSH1)	4	1	0	0	5	es 12.01	p=0.02
GA_1529	NM_172164	nuclear autoantigenic sperm protein (histone-binding) (NASP), transcript variant 1	58	7	32	15	112	es 3.23	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_1634	NM_002647	phosphoinositide-3-kinase, class 3 (PIK3C3)	5	1	1	2	9	es 3.75	p=0.05
GA_1650	NM_002660	phospholipase C, gamma 1 (formerly subtype 148) (PLCG1)	10	4	4	1	19	es 3.34	p=0.01
GA_1662	AF195139	pinin (PINN) gene, complete cds	23	9	7	5	44	es 3.29	p=0.00
GA_1665	NM_002691	polymerase (DNA directed), delta 1, catalytic subunit 125kDa (POLD1)	9	6	2	1	18	es 3.00	p=0.02
GA_1674	NM_002701	POU domain, class 5, transcription factor 1 (POU5F1)	24	1	2	0	27	es 24.03	p=0.00
GA_1696	NM_000947	primase, polypeptide 2A, 58kDa (PRIM2A)	4	0	0	1	5	es 12.01	p=0.02
GA_1702	NM_002740	protein kinase C, iota (PRKCI)	8	2	2	1	13	es 4.81	p=0.01
GA_171	BC013923	Similar to SRY-box containing gene 2 sequence	12	1	1	3	17	es 7.21	p=0.00
GA_1710	NM_002764	phosphoribosyl pyrophosphate synthetase 1 (PRPS1)	7	3	2	1	13	es 3.50	p=0.02
GA_1752	NM_152881	PTK7 protein tyrosine kinase 7 (PTK7), transcript variant 3	15	14	5	3	37	es 2.05	p=0.04
GA_1777	NM_002862	phosphorylase, glycogen; brain (PYGB), nuclear gene encoding mitochondrial protein	13	8	1	2	24	es 3.55	p=0.00
GA_1794	NM_003610	RAE1 RNA export 1 homolog (S. pombe) (RAE1)	5	0	0	2	7	es 7.51	p=0.01
GA_1814	NM_002907	RecQ protein-like (DNA helicase Q1-like) (RECQL), transcript variant 1	4	2	0	0	6	es 6.01	p=0.04
GA_1820	NM_002916	replication factor C (activator 1) 4, 37kDa (RFC4)	6	0	2	2	10	es 4.51	p=0.02
GA_1865	NM_002949	mitochondrial ribosomal protein L12 (MRPL12), nuclear gene encoding mitochondrial protein	4	0	0	2	6	es 6.01	p=0.04

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression
			ES	EB	preHEP	preNeu	Total
		nuclear gene encoding mitochondrial protein					
GA_1909	NM_003012	secreted frizzled-related protein 1 (SFRP1)	12	8	1	7	28
							es 2.25
GA_1938	NM_003601	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5 (SMARCA5)	19	10	4	5	38
							es 3.00
GA_1942	NM_003076	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1 (SMARCD1), transcript variant 1	10	3	3	3	19
							es 3.34
GA_1962	NM_152826	sorting nexin 1 (SNX1), transcript variant 3	4	0	0	1	5
							es 12.01
GA_1963	NM_003100	sorting nexin 2 (SNX2)	8	2	4	1	15
							es 3.43
GA_2024	NM_003212	teratocarcinoma-derived growth factor 1 (TDGF1)	20	1	0	0	21
							es 60.07
GA_2031	NM_003234	transferrin receptor (p90, CD71) (TFR)	13	9	3	4	29
							es 2.44
GA_2066	NM_003283	troponin T1, skeletal, slow (TNNT1)	5	1	1	0	7
							es 7.51
GA_2091	NM_001069	tubulin, beta polypeptide (TUBB)	40	13	11	17	81
							es 2.93
GA_2123	NM_003481	ubiquitin specific protease 5 (isopeptidase T) (USP5)	13	6	5	1	25
							es 3.25
GA_2149	NM_003413	Zic family member 3 heterotaxy 1 (odd-paired homolog, Drosophila) (ZIC3)	7	0	1	0	8
							es 21.02
GA_2175	NM_001605	alanyl-tRNA synthetase (AARS)	23	6	1	3	33
							es 6.91
GA_2178	NM_001104	actinin, alpha 3 (ACTN3)	6	1	0	0	7
							es 18.02
GA_2234	NM_000107	damage-specific DNA binding protein 2, 48kDa (DDB2)	8	1	0	2	11
							es 8.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
		(DDB2)						
GA_2235	NM_001358	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 15 (DDX15)	13	7	3	1	24	es 3.55 p=0.00
GA_2240	NM_001384	diphtheria toxin resistance protein required for diphthamide biosynthesis-like 2 (S. cerevisiae) (DPH2L2)	6	1	2	0	9	es 6.01 p=0.01
GA_2271	NM_001533	heterogeneous nuclear ribonucleoprotein L (HNRPL)	10	1	4	5	20	es 3.00 p=0.02
GA_2289	NM_000234	ligase I, DNA, ATP-dependent (LIG1)	10	2	5	3	20	es 3.00 p=0.02
GA_2319	NM_000456	sulfite oxidase (SUOX), nuclear gene encoding mitochondrial protein	5	1	1	0	7	es 7.51 p=0.01
GA_2323	NM_002164	indoleamine-pyrrole 2,3 dioxygenase (INDO)	6	0	0	0	6	es >4 p=0.00
GA_2334	NM_000216	Kallmann syndrome 1 sequence (KAL1)	5	0	1	0	6	es 15.02 p=0.00
GA_2337	NM_003501	acyl-Coenzyme A oxidase 3, pristanoyl (ACOX3)	4	0	0	1	5	es 12.01 p=0.02
GA_23430	NM_006474	lung type-I cell membrane-associated glycoprotein (T1A-2)	5	2	1	0	8	es 5.01 p=0.03
GA_23457	AK055600	cDNA FLJ1038 fis, clone HSYRA2000159 sequence	6	2	0	2	10	es 4.51 p=0.02
GA_23467	AK092578	cDNA FLJ35259 fis, clone PROST2004251 sequence	4	0	0	0	4	es >4 p=0.00
GA_23468			6	2	0	2	10	es 4.51 p=0.02
GA_23476			5	0	2	0	7	es 7.51 p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression
			ES	EB	preHEP	preNeu	Total
GA_23484			43	0	1	0	44
GA_23485			25	1	1	0	27
GA_23486			7	0	0	0	7
GA_23487			49	0	0	0	49
GA_23488			9	0	0	0	9
GA_23489			13	0	0	0	13
GA_23490			12	1	1	0	14
GA_23514			5	1	0	2	8
GA_23515			4	0	0	0	4
GA_23525			8	3	0	0	11
GA_2356	NM_002851	protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1)	10	0	0	0	10
GA_2357	NM_001670	armadillo repeat gene deletes in velocardiofacial syndrome (ARVCF)	6	0	0	0	6
GA_23572			4	1	1	0	6
GA_23577			4	2	0	0	6
GA_23578	BM454360	AGENCOURT_6402318 NIH_MGC_85cDNA clone IMAGE:5497491 5' sequence	6	0	0	0	6
GA_23579			4	0	0	0	4

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression
			ES	EB	preHEP	preNeu	Total
GA_23585			8	0	1	1	10
GA_23596			4	0	1	0	5
GA_23612	NM_005762	tripartite motif-containing 28 protein; KRAB-associated protein 1; transcriptional intermediary factor 1-beta; nuclear corepressor KAP-1 sequence	6	2	1	0	9
GA_23615			4	1	0	0	5
GA_23634			4	1	0	0	5
GA_2367	NM_003923	forkhead box H1 (FOXH1)	5	0	0	0	5
GA_23673			5	1	0	0	6
GA_23683			4	1	1	0	6
GA_23981	AK057602	cDNA FLJ33040 fis, clone THYMU2000382, weakly similar to 60S RIBOSOMAL PROTEIN L12	4	0	0	0	4
GA_2418	NM_004317	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial) (ASNA1)	6	3	1	1	11
GA_2436	NM_004329	bone morphogenetic protein receptor, type Ia (BMPRI1A)	7	3	1	1	12
GA_2442	NM_004335	bone marrow stromal cell antigen 2 (BST2)	13	0	2	3	18
GA_2443	NM_004336	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast) (BUB1)	10	5	4	2	21
GA_2444	NM_004725	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast) (BUB3)	12	4	7	4	27

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression
			ES	EB	preHEP	preNeu	Total
GA_2447	NM_004341	carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), nuclear gene encoding mitochondrial protein	11	8	2	1	22
GA_2467	NM_004804	WD40 protein Ciao1 (CIAO1)	8	0	1	2	11
GA_2496	NM_004229	cofactor required for Sp1 transcriptional activation, subunit 2, 150kDa (CRSP2)	7	1	1	2	11
GA_2501	NM_080598	HLA-B associated transcript 1 (BAT1), transcript variant 2	24	13	13	9	59
GA_2621	NM_004135	isocitrate dehydrogenase 3 (NAD+) gamma (IDH3G)	5	2	0	1	8
GA_2641	NM_017522	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor (LRP8), transcript variant 3	7	0	0	2	9
GA_2643	NM_004635	mitogen-activated protein kinase-activated protein kinase 3 (MAPKAPK3)	6	0	1	2	9
GA_2644	NM_004526	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae) (MCM2)	23	8	6	4	41
GA_2717	NM_004703	rabaptin-5 (RAB5EP)	5	1	1	0	7
GA_2728	NM_004168	succinate dehydrogenase complex, subunit A, flavoprotein (Fp) (SDHA), nuclear gene encoding mitochondrial protein	5	2	0	2	9
GA_2751	NM_004596	small nuclear ribonucleoprotein polypeptide A (SNRPA)	11	3	4	5	23
GA_2762	NM_004819	sympleskin; Huntingtin interacting protein I (SPK)	10	5	6	1	22
GA_2784	NM_004818	prp28, U5 snRNP 100 kd protein (U5-100K)	16	14	3	3	36

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_2791	NM_004652	ubiquitin specific protease 9, X chromosome (fat	10	2	2	1	15	
GA_2800	NM_004629	facets-like Drosophila) (USP9X), transcript variant 1	5	0	2	1	8	es 6.01 p=0.00
GA_2840	NM_004960	Fanconi anemia, complementation group G (FANCG)	14	2	4	1	21	es 5.01 p=0.03
GA_2857	NM_004987	fusion, derived from t(12;16) malignant liposarcoma (FUS)	5	2	0	1	8	es 6.01 p=0.00
GA_2868	NM_005006	LIM and senescent cell antigen-like domains 1 (LIMS1)	6	1	2	2	11	es 5.01 p=0.03
GA_2889	NM_005032	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase) (NDUFS1)	35	18	7	19	79	es 3.60 p=0.03
GA_2897	NM_005044	plastin 3 (T isoform) (PLS3)	6	3	0	1	10	es 2.39 p=0.00
GA_2898	NM_005049	protein kinase, X-linked (PRKX)	6	0	1	2	9	es 4.51 p=0.02
GA_2937	NM_005207	PWP2 periodic tryptophan protein homolog (yeast) (PWP2H)	6	1	0	0	7	es 6.01 p=0.01
GA_2945	NM_005232	v-crk sarcoma virus CT10 oncogene homolog (avian)-like (CRKL)	5	1	1	1	8	es 18.02 p=0.00
GA_2962	NM_005314	Epha1 (EPHA1)	4	0	0	0	4	es 5.01 p=0.03
GA_2984	NM_005474	gastrin-releasing peptide receptor (GRPR)	6	4	1	0	11	es >4 p=0.00
GA_2988	NM_005397	histone deacetylase 5 (HDAC5), transcript variant 1	59	23	5	8	95	es 3.60 p=0.03
GA_3017	NM_000098	podocalyxin-like (PODXL)	4	1	1	0	6	es 4.92 p=0.00
		caritine palmitoyltransferase II (CPT2), nuclear gene encoding mitochondrial protein					es 6.01	p=0.04

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Geron ID	GenBank ID	Name		EST counts				Relative Expression		
				ES	EB	preHEP	preNeu		Total	
GA_3024	NM_003902	far upstream element (FUSE) binding protein 1 (FUBP1)	⊗	13	4	6	3	26	es 3.00	p=0.01
GA_3042	NM_005760	CCAAT-box-binding transcription factor (CBF2)	⊗	9	2	2	3	16	es 3.86	p=0.01
GA_3055	NM_005864	signal transduction protein (SH3 containing) (EFS2), transcript variant 1		6	1	0	1	8	es 9.01	p=0.00
GA_3112	NM_005789	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki) (PSME3)		12	2	6	2	22	es 3.60	p=0.00
GA_3118	NM_005778	RNA binding motif protein 5 (RBM5)		11	6	4	4	25	es 2.36	p=0.04
GA_3130	NM_005785	hypothetical SBB103 protein (SBB103)		4	1	0	0	5	es 12.01	p=0.02
GA_3134	NM_005877	splicing factor 3a, subunit 1, 120kDa (SF3A1)		10	1	4	3	18	es 3.75	p=0.01
GA_3137	NM_005628	solute carrier family 1 (neutral amino acid transporter), member 5 (SLC1A5)		23	11	2	13	49	es 2.66	p=0.00
GA_3144	NM_005839	serine/arginine repetitive matrix 1 (SRRM1)		16	6	5	8	35	es 2.53	p=0.01
GA_3150	NM_139315	TAF6 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 80kDa (TAF6), transcript variant 2		4	0	0	0	4	es >4	p=0.00
GA_3175	NM_005741	zinc finger protein 263 (ZNF263)	⊗	7	4	0	1	12	es 4.20	p=0.01
GA_3178	NM_006017	prominin-like 1 (mouse) (PROML1)		7	2	2	0	11	es 5.26	p=0.01
GA_3183	NM_006035	CDC42 binding protein kinase beta (DMPK-like) (CDC42BPB)		13	5	0	3	21	es 4.88	p=0.00
GA_3219	NM_005928	milk fat globule-EGF factor 8 protein (MFGE8)		30	11	11	14	66	es 2.50	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_32806	BE568403	601341979F1 NIH_MGC_53cDNA clone IMAGE:3684283 5' sequence	9	2	5	2	18	es 3.00	p=0.02
GA_32836	AK055259	cDNA FLJ30697 fis, clone FCBBF2000815, weakly similar to ZYXIN	4	0	1	1	6	es 6.01	p=0.04
GA_32842			8	3	0	0	11	es 8.01	p=0.00
GA_32860			7	0	0	0	7	es >4	p=0.00
GA_32868	AK091598	cDNA FLJ34279 fis, clone FEBRA2003833 sequence	4	0	0	0	4	es >4	p=0.00
GA_32887	NM_006141	dynein, cytoplasmic, light intermediate polypeptide 2 (DNCL12)	7	2	0	2	11	es 5.26	p=0.01
GA_32895			5	4	0	0	9	es 3.75	p=0.05
GA_32908	AL832758	mRNA; cDNA DKFZp686C0927 (from clone DKFZp686C0927) sequence	4	0	0	0	4	es >4	p=0.00
GA_32913			4	0	0	0	4	es >4	p=0.00
GA_32917			4	0	0	0	4	es >4	p=0.00
GA_32926			7	0	0	0	7	es >4	p=0.00
GA_32947			4	0	2	0	6	es 6.01	p=0.04
GA_32979			4	0	0	0	4	es >4	p=0.00
GA_32985			4	0	0	0	4	es >4	p=0.00
GA_3321	NM_006345	chromosome 4 open reading frame 1 (C4orf1)	10	5	4	2	21	es 2.73	p=0.02

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_33423	NM_002537	ornithine decarboxylase antizyme 2 (OAZ2)	18	1	7	3	29	es 4.91	p=0.00
GA_3343	NM_006392	nucleolar protein 5A (56kDa with KKE/D repeat) (NOL5A)	16	5	11	5	37	es 2.29	p=0.02
GA_33455	NM_006047	RNA binding motif protein 12 (RBM12), transcript variant 1	17	4	3	4	28	es 4.64	p=0.00
GA_33475	NM_004902	RNA-binding region (RNP1, RRM) containing 2 (RNPC2)	12	2	8	2	24	es 3.00	p=0.01
GA_33503	NM_018135	mitochondrial ribosomal protein S18A (MRPS18A), nuclear gene encoding mitochondrial protein	4	1	1	0	6	es 6.01	p=0.04
GA_33528	NM_032803	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3 (SLC7A3)	4	0	1	0	5	es 12.01	p=0.02
GA_33533	BC037428	Unknown (protein for MGC:46327) sequence	7	4	1	1	13	es 3.50	p=0.02
GA_33548	NM_015638	chromosome 20 open reading frame 188 (C20orf188)	7	3	0	1	11	es 5.26	p=0.01
GA_33588	AL832967	mRNA; cDNA DKFZp666B082 (from clone DKFZp666B082) sequence	5	0	2	1	8	es 5.01	p=0.03
GA_33680	NM_016089	KRAB-zinc finger protein SZF1-1 (SZF1)	⊗ 15	0	1	0	16	es 45.05	p=0.00
GA_33684	NM_005186	calpain 1, (mu/l) large subunit (CAPN1)	13	8	1	5	27	es 2.79	p=0.01
GA_33691	AL117507	mRNA; cDNA DKFZp434F1935 (from clone DKFZp434F1935); partial cds	4	1	1	0	6	es 6.01	p=0.04
GA_33704	AL833549	mRNA; cDNA DKFZp686N183 (from clone DKFZp686N183) sequence	4	1	1	0	6	es 6.01	p=0.04
GA_33730	AL832779	mRNA; cDNA DKFZp686H157 (from clone DKFZp686H157) sequence	4	0	1	1	6	es 6.01	p=0.04

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_33747	NM_032737	lamin B2 (LMNB2)	11	8	3	3	25	es 2.36 p=0.04
GA_33755	NM_033547	hypothetical gene MGC16733 similar to CG12113 (MGC16733)	5	0	0	1	6	es 15.02 p=0.00
GA_33772	BF223023	7q27f09.x1 NCLCGAP_GC6cDNA clone IMAGE:3699616 3' sequence	5	0	0	0	5	es >4 p=0.00
GA_33816	NM_015850	fibroblast growth factor receptor 1 (frms-related tyrosine kinase 2, Pfeiffer syndrome) (FGFR1), transcript variant 2	35	12	9	5	61	es 4.04 p=0.00
GA_33874	NM_017730	hypothetical protein FLJ20259 (FLJ20259)	19	6	4	4	33	es 4.08 p=0.00
GA_33876	NM_148904	oxysterol binding protein-like 9 (OSBPL9), transcript variant 1	5	1	0	2	8	es 5.01 p=0.03
GA_33877	NM_020796	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A (SEMA6A)	16	1	11	4	32	es 3.00 p=0.00
GA_33959	NM_030964	sprouty homolog 4 (Drosophila) (SPRY4)	4	1	0	0	5	es 12.01 p=0.02
GA_34010	AK000089	cDNA FLJ20082 fis, clone COL03245	8	0	3	0	11	es 8.01 p=0.00
GA_34047	NM_170752	chromodomain protein, Y chromosome-like (CDYL), transcript variant 3	8	1	1	1	11	es 8.01 p=0.00
GA_34061	NM_152429	hypothetical protein MGC39320 (MGC39320)	7	1	0	1	9	es 10.51 p=0.00
GA_3407	NM_006328	RNA binding motif protein 14 (RBM14)	16	3	4	3	26	es 4.81 p=0.00
GA_34077	NM_133457	likely ortholog of mouse type XXVI collagen (COL26A1)	7	0	4	2	13	es 3.50 p=0.02
GA_34137	NM_020314	esophageal cancer associated protein (MGC16824)	6	1	0	0	7	es 18.02 p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_34200	NM_005763	aminoadipate-semialdehyde synthase (AASS)	10	0	0	2	12	es 15.02	p=0.00
GA_34219	NM_018449	ubiquitin associated protein 2 (UBAP2), transcript variant 1	6	2	1	0	9	es 6.01	p=0.01
GA_34245	NM_004922	SEC24 related gene family, member C (S. cerevisiae) (SEC24C)	10	6	0	1	17	es 4.29	p=0.00
GA_34270	NM_152758	hypothetical protein FLJ31657 (FLJ31657)	5	2	1	0	8	es 5.01	p=0.03
GA_34280	NM_000702	ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide (ATP1A2)	4	0	0	0	4	es >4	p=0.00
GA_34320	NM_006461	sperm associated antigen 5 (SPAG5)	14	6	5	2	27	es 3.23	p=0.00
GA_34322	NM_023926	hypothetical protein FLJ12895 (FLJ12895)	5	0	1	2	8	es 5.01	p=0.03
GA_3436	NM_018062	hypothetical protein FLJ10335 (FLJ10335)	5	1	3	0	9	es 3.75	p=0.05
GA_34419	NM_002952	ribosomal protein S2 (RPS2)	19	5	11	7	42	es 2.48	p=0.00
GA_34438	NM_006521	transcription factor binding to IGHM enhancer 3 (TFE3)	5	2	0	2	9	es 3.75	p=0.05
GA_34480	NM_012218	interleukin enhancer binding factor 3, 90kDa (ILF3), transcript variant 1	41	26	13	20	100	es 2.09	p=0.00
GA_34503	NM_005762	tripartite motif-containing 28 (TRIM28)	13	6	8	2	29	es 2.44	p=0.02
GA_34505	NM_002065	glutamate-ammonia ligase (glutamine synthase) (GLUL)	21	1	8	2	32	es 5.73	p=0.00
GA_34522	NM_000071	cystathionine-beta-synthase (CBS)	7	2	1	2	12	es 4.20	p=0.01
GA_34539	NM_002880	v-raf-1 murine leukemia viral oncogene homolog 1 (RAF1)	14	7	3	0	24	es 4.20	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_34563	NM_007192	suppressor of Ty 16 homolog (<i>S. cerevisiae</i>) (SUPT16H)	9	1	1	3	14	es 5.41	p=0.00
GA_34594	NM_004426	polyhomeotic-like 1 (<i>Drosophila</i>) (PHC1) ⊗	6	0	0	0	6	es >4	p=0.00
GA_34606	NM_015570	autism susceptibility candidate 2 (AUTS2)	7	0	0	2	9	es 10.51	p=0.00
GA_34626	NM_004911	protein disulfide isomerase related protein (calcium-binding protein, intestinal-related) (ERP70)	5	2	1	1	9	es 3.75	p=0.05
GA_34655	X74794	P1 Cdc21 protein sequence	34	9	5	4	52	es 5.67	p=0.00
GA_34679	NM_002015	forkhead box O1A (rhabdomyosarcoma) (FOXO1A) ⊗	4	0	1	1	6	es 6.01	p=0.04
GA_34715	NM_002421	matrix metalloproteinase 1 (interstitial collagenase) (MMP1)	5	1	0	2	8	es 5.01	p=0.03
GA_34820	NM_024656	hypothetical protein FLJ22329 (FLJ22329)	5	1	1	1	8	es 5.01	p=0.03
GA_34875	NM_004459	fetal Alzheimer antigen (FALZ)	5	2	0	2	9	es 3.75	p=0.05
GA_35037	NM_004426	polyhomeotic-like 1 (<i>Drosophila</i>) (PHC1) ⊗	34	3	2	5	44	es 10.21	p=0.00
GA_35125	NM_005386	neuronatin (NNAT)	5	3	0	1	9	es 3.75	p=0.05
GA_35141	NM_018555	zinc finger protein 331; zinc finger protein 463 (ZNF361) ⊗	13	2	5	2	22	es 4.34	p=0.00
GA_35150	AB014542	KIAA0642 protein sequence	5	1	2	1	9	es 3.75	p=0.05
GA_35158	NM_015327	KIAA1089 protein (KIAA1089)	10	6	2	2	20	es 3.00	p=0.02
GA_3520	NM_005915	MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, <i>S. pombe</i>) (<i>S. cerevisiae</i>) (MCM6)	12	5	5	2	24	es 3.00	p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression			
			ES	EB	preHEP	preNeu		Total		
GA_35206	NM_005678	SNRPN upstream reading frame (SNURF), transcript variant 1	20	10	9	9	48	es 2.15	p=0.01	
GA_35221	NM_020442	KIAA1885 protein (DKFZP434L1435)	6	0	0	0	0	6	es >4	p=0.00
GA_35231	NM_014389	proline and glutamic acid rich nuclear protein (PELP1)	14	11	3	1	1	29	es 2.80	p=0.01
GA_35233	NM_138615	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 30 (DDX30), transcript variant 1	11	3	4	5	5	23	es 2.75	p=0.02
GA_35239	NM_014633	KIAA0155 gene product (KIAA0155)	5	1	2	0	0	8	es 5.01	p=0.03
GA_35260	NM_004104	fatty acid synthase (FASN)	6	2	0	1	1	9	es 6.01	p=0.01
GA_35393	NM_006861	RAB35, member RAS oncogene family (RAB35)	7	2	2	1	1	12	es 4.20	p=0.01
GA_35395	NM_024662	hypothetical protein FLJ10774 (FLJ10774)	6	4	0	1	1	11	es 3.60	p=0.03
GA_35405			12	8	3	1	1	24	es 3.00	p=0.01
GA_35422	NM_021211	transposon-derived Buster1 transposase-like protein (LOC58486)	4	0	0	2	2	6	es 6.01	p=0.04
GA_35457	AJ459424	JEMMA protein sequence	7	1	2	1	1	11	es 5.26	p=0.01
GA_35481	NM_006452	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS)	36	14	13	9	9	72	es 3.00	p=0.00
GA_35495	NM_003472	DEK oncogene (DNA binding) (DEK) ⊗	16	3	8	10	10	37	es 2.29	p=0.02
GA_35547	NM_032202	hypothetical protein KIAA1109 (KIAA1109)	4	0	0	2	2	6	es 6.01	p=0.04
GA_35558	AL831917	hypothetical protein sequence	6	1	1	1	1	9	es 6.01	p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_3559	NM_005629	solute carrier family 6 (neurotransmitter transporter, creatine), member 8 (SLC6A8)	5	1	0	1	7	es 7.51 p=0.01
GA_35606	NM_024586	oxysterol binding protein-like 9 (OSBPL9), transcript variant 6	4	1	1	0	6	es 6.01 p=0.04
GA_35607	AB002366	KIAA0368 sequence	8	4	2	3	17	es 2.67 p=0.05
GA_35615	NM_000251	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli) (MSH2)	16	6	6	0	28	es 4.00 p=0.00
GA_35687	NM_033502	transcriptional regulating protein 132 (TRP-132), transcript variant 1	5	0	0	0	5	es >4 p=0.00
GA_35693	NM_014782	armadillo repeat protein ALEX2 (ALEX2)	12	8	4	3	27	es 2.40 p=0.03
GA_35762	NM_020765	retinoblastoma-associated factor 600 (RBAF600)	12	4	3	1	20	es 4.51 p=0.00
GA_35833	NM_015878	ornithine decarboxylase antizyme inhibitor (OAZIN), transcript variant 1	17	8	10	6	41	es 2.13 p=0.02
GA_35852	AK056479	cDNA FLJ31917 fis, clone NT2RP7004925, weakly similar to VASODILATOR-STIMULATED PHOSPHOPROTEIN	4	2	0	0	6	es 6.01 p=0.04
GA_35869	AB011112	KIAA0540 protein sequence	5	2	1	0	8	es 5.01 p=0.03
GA_35905	NM_006640	MLL septin-like fusion (MSF)	28	25	6	6	65	es 2.27 p=0.00
GA_35913	NM_018265	hypothetical protein FLJ10901 (FLJ10901)	5	0	1	1	7	es 7.51 p=0.01
GA_3593	NM_000270	nucleoside phosphorylase (NP)	5	1	1	1	8	es 5.01 p=0.03
GA_35955	NM_022754	sideroflexin 1 (SFXN1)	5	1	1	0	7	es 7.51 p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_35984	NM_015340	leucyl-tRNA synthetase, mitochondrial (LARS2), nuclear gene encoding mitochondrial protein	4	0	2	0	6	es 6.01	p=0.04
GA_36015	NM_015341	barren homolog (Drosophila) (BRRN1)	9	1	1	2	13	es 6.76	p=0.00
GA_36017	AK074137	FLJ00210 protein sequence	4	0	1	0	5	es 12.01	p=0.02
GA_36019	NM_012426	splicing factor 3b, subunit 3, 130kDa (SF3B3)	11	3	2	3	19	es 4.13	p=0.00
GA_36080	NM_152333	chromosome 14 open reading frame 69 (C14orf69)	14	1	1	3	19	es 8.41	p=0.00
GA_36090	NM_020444	KIAA1191 protein (KIAA1191)	9	7	1	2	19	es 2.70	p=0.03
GA_3611	NM_001211	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast) (BUB1B)	13	4	4	4	25	es 3.25	p=0.00
GA_36126	NM_004286	GTP binding protein 1 (GTPBP1)	4	1	0	0	5	es 12.01	p=0.02
GA_36127	NM_016121	NY-REN-45 antigen (NY-REN-45)	5	1	2	1	9	es 3.75	p=0.05
GA_36129	NM_018353	hypothetical protein FLJ11186 (FLJ11186)	10	0	3	3	16	es 5.01	p=0.00
GA_36133	NM_020428	CTL2 gene (CTL2)	9	6	0	0	15	es 4.51	p=0.00
GA_36137	NM_007363	non-POU domain containing, octamer-binding (NONO)	39	12	22	14	87	es 2.44	p=0.00
GA_36139	NM_004990	methionine-tRNA synthetase (MARS)	11	3	1	0	15	es 8.26	p=0.00
GA_36155	AB020719	KIAA0912 protein sequence	5	1	1	0	7	es 7.51	p=0.01
GA_36183	NM_016333	serine/arginine repetitive matrix 2 (SRRM2)	23	21	9	1	54	es 2.23	p=0.00
GA_36184	NM_020151	START domain containing 7 (STARD7), transcript variant 1	17	6	0	1	24	es 7.29	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
		variant 1						
GA_36219	NM_152392	hypothetical protein DKFZp564C236 (DKFZp564C236)	7	1	2	1	11	es 5.26 p=0.01
GA_36221	NM_000966	retinoic acid receptor, gamma (RARG)	6	2	0	2	10	es 4.51 p=0.02
GA_36241	NM_018031	WD repeat domain 6 (WDR6), transcript variant 1	29	20	11	7	67	es 2.29 p=0.00
GA_36270	NM_003715	vesicle docking protein p115 (VDP)	12	5	4	2	23	es 3.28 p=0.01
GA_3628	NM_006579	emopamil binding protein (sterol isomerase) (EBP)	7	1	3	0	11	es 5.26 p=0.01
GA_36307	NM_015897	protein inhibitor of activated STAT protein PIASy (PIASY)	5	2	2	0	9	es 3.75 p=0.05
GA_36389	NM_025256	HLA-B associated transcript 8 (BAT8), transcript variant NG36/G9a-SPI	11	5	6	2	24	es 2.54 p=0.03
GA_36450	NM_003051	solute carrier family 16 (monocarboxylic acid transporters), member 1 (SLC16A1)	22	7	7	5	41	es 3.48 p=0.00
GA_36474	X87832	NOV	5	4	0	0	9	es 3.75 p=0.05
GA_36491	NM_024611	similar to NMDA receptor-regulated gene 2 (mouse) (FLJ11896)	6	4	0	1	11	es 3.60 p=0.03
GA_36526	NM_033557	similar to putative transmembrane protein; homolog of yeast Golgi membrane protein Yif1p (Yif1p-interacting factor) (LOC90522)	6	3	2	0	11	es 3.60 p=0.03
GA_36545	AB014600	KIAA0700 protein sequence	8	4	1	3	16	es 3.00 p=0.04
GA_36581	NM_018071	hypothetical protein FLJ10357 (FLJ10357)	6	3	0	0	9	es 6.01 p=0.01

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Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_36592	AB002363	KIAA0365 sequence	6	1	0	1	8	es 9.01	p=0.00
GA_36595	NM_024718	hypothetical protein FLJ10101 (FLJ10101)	8	4	2	3	17	es 2.67	p=0.05
GA_36643	NM_003918	glycogenin 2 (GYG2)	5	1	0	0	6	es 15.02	p=0.00
GA_36675	NM_003605	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase) (OGT)	9	4	0	1	14	es 5.41	p=0.00
GA_36692	NM_015902	progesterin induced protein (DD5)	8	4	1	2	15	es 3.43	p=0.02
GA_36707	NM_021627	sentrin-specific protease (SENP2)	4	0	1	0	5	es 12.01	p=0.02
GA_36730	AF164609	endogenous retrovirus HERV-K101, complete sequence	5	0	0	0	5	es >4	p=0.00
GA_36734	AF376802	neuroligin 2 sequence	6	3	0	0	9	es 6.01	p=0.01
GA_36771	NM_016238	anaphase-promoting complex subunit 7 (ANAPC7)	6	0	1	0	7	es 18.02	p=0.00
GA_36788	NM_000141	fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome) (FGFR2), transcript variant 1	9	5	1	2	17	es 3.38	p=0.02
GA_36798	NM_000071	cystathionine-beta-synthase (CBS)	11	0	1	2	14	es 11.01	p=0.00
GA_36842	NM_006197	pericentriolar material 1 (PCM1)	6	3	1	1	11	es 3.60	p=0.03
GA_36897	NM_006773	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 18 (Myc-regulated) (DDX18)	7	3	2	1	13	es 3.50	p=0.02

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Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_36933	NM_016424	cisplatin resistance-associated overexpressed protein (LUC7A)	19	1	4	7	31	es 4.76 p=0.00
GA_36936	NM_149379	Williams Beuren syndrome chromosome region 20C (WBSCR20C), transcript variant 4	11	6	4	1	22	es 3.00 p=0.01
GA_36951	NM_005916	MCM7 minichromosome maintenance deficient 7 (S. cerevisiae) (MCM7)	19	3	6	11	39	es 2.85 p=0.00
GA_36957	NM_024642	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetyl/galactosaminyltransferase 12 (GalNAc-T12) (GALNT12)	4	0	1	1	6	es 6.01 p=0.04
GA_36964	NG_001332	T cell receptor alpha delta locus (TCRA/TCRD) on chromosome 14	16	2	0	0	18	es 24.03 p=0.00
GA_36974	AL834155	mRNA; cDNA DKFZp761O0611 (from clone DKFZp761O0611) sequence	4	1	0	1	6	es 6.01 p=0.04
GA_36977	NM_020927	KIAA1576 protein (KIAA1576)	9	2	1	0	12	es 9.01 p=0.00
GA_37071	NM_153759	DNA (cytosine-5-)-methyltransferase 3 alpha (DNMT3A), transcript variant 2	9	2	1	1	13	es 6.76 p=0.00
GA_37078	NM_014977	apoptotic chromatin condensation inducer in the nucleus (ACINUS)	10	6	2	2	20	es 3.00 p=0.02
GA_37079	NM_032156	EEG1 (EEG1), transcript variant S	7	0	0	0	7	es >4 p=0.00
GA_37094	AL832758	mRNA; cDNA DKFZp686C0927 (from clone DKFZp686C0927) sequence	11	1	3	3	18	es 4.72 p=0.00
GA_37215	NM_019023	hypothetical protein FLJ10640 (FLJ10640)	7	1	3	0	11	es 5.26 p=0.01
GA_3723	NM_003750	eukaryotic translation initiation factor 3, subunit 10 theta, 150/170kDa (EIF3S10)	30	15	6	17	68	es 2.37 p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_37251	NM_000604	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome) (FGFR1), transcript variant 1	7	1	5	0	13	es 3.50	p=0.02
GA_3730	NM_003751	eukaryotic translation initiation factor 3, subunit 9 eta, 116kDa (EIF3S9)	13	5	2	3	23	es 3.90	p=0.00
GA_37314	NM_003169	suppressor of Ty 5 homolog (S. cerevisiae) (SUPT5H)	14	6	1	1	22	es 5.26	p=0.00
GA_37354	NM_015726	H326 (H326)	5	1	1	0	7	es 7.51	p=0.01
GA_37372	NM_024658	importin 4 (FLJ23338)	12	7	0	3	22	es 3.60	p=0.00
GA_37389	NM_017647	FtsJ homolog 3 (E. coli) (FTSJ3)	13	7	5	1	26	es 3.00	p=0.01
GA_37391	NM_004938	death-associated protein kinase 1 (DAPK1)	6	0	0	1	7	es 18.02	p=0.00
GA_37399	NM_148842	Williams-Beuren syndrome chromosome region 16 (WBSCR16), transcript variant 2	10	0	1	2	13	es 10.01	p=0.00
GA_37409	NM_021145	cyclin D binding myb-like transcription factor 1 (DMTF1)	5	1	0	2	8	es 5.01	p=0.03
GA_37424	NM_152742	hypothetical protein DKFZp547M109 (DKFZp547M109)	6	0	1	2	9	es 6.01	p=0.01
GA_37431	NM_006034	p53-induced protein (PIG11)	7	4	1	0	12	es 4.20	p=0.01
GA_37478	NM_014670	basic leucine zipper and W2 domains 1 (BZW1)	24	13	11	9	57	es 2.18	p=0.01
GA_37504	NM_153613	PI3C domain containing hypothetical protein (LOC254531)	5	1	0	3	9	es 3.75	p=0.05
GA_37536	AK026970	cDNA: FLJ23317 fis, clone HEP12062, highly similar to AF008936syntaxin-16B mRNA	5	2	1	0	8	es 5.01	p=0.03

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu	Total		
GA_37538	NM_080797	death associated transcription factor 1 (DATF1), transcript variant 3	6	0	1	0	7	es 18.02	p=0.00
GA_37589	AL834216	hypothetical protein sequence	4	0	1	0	5	es 12.01	p=0.02
GA_37595	NM_015062	KIAA0595 protein (KIAA0595)	7	3	0	1	11	es 5.26	p=0.01
GA_37606	NM_019012	phosphoinositol 3-phosphate-binding protein-2 (PEPP2)	4	2	0	0	6	es 6.01	p=0.04
GA_37707	NM_022574	PERQ amino acid rich, with GYF domain 1 (PERQ1)	4	0	1	0	5	es 12.01	p=0.02
GA_37729	NM_005436	DNA segment on chromosome 10 (unique) 170 (D10S170)	8	4	1	3	16	es 3.00	p=0.04
GA_37737	NM_003707	RuvB-like 1 (E. coli) (RUVBL1)	5	2	0	2	9	es 3.75	p=0.05
GA_37755	NM_015044	golgi associated, gamma adaptin ear containing, ARF binding protein 2 (GGA2), transcript variant 1	13	5	0	2	20	es 5.58	p=0.00
GA_37788	NM_133631	roundabout, axon guidance receptor, homolog 1 (Drosophila) (ROBO1), transcript variant 2	7	4	1	0	12	es 4.20	p=0.01
GA_37800	NM_032701	hypothetical protein MGC2705 (MGC2705)	4	1	0	1	6	es 6.01	p=0.04
GA_37805	NM_025222	hypothetical protein PRO2730 (PRO2730)	6	1	3	1	11	es 3.60	p=0.03
GA_37866	NM_138927	SON DNA binding protein (SON), transcript variant f	6	3	2	0	11	es 3.60	p=0.03
GA_37877	NM_012215	meningioma expressed antigen 5 (hyaluronidase) (MGEA5)	10	4	3	3	20	es 3.00	p=0.02
GA_37884	AB032993	KIAA1167 protein sequence	5	2	1	0	8	es 5.01	p=0.03
GA_37904	NM_000478	alkaline phosphatase, liver/bone/kidney (ALPL)	4	1	1	0	6	es 6.01	p=0.04

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_37914	NM_153464	interleukin enhancer binding factor 3, 90kDa (ILF3), $\otimes$ transcript variant 3	9	1	1	0	11	es 13.52	p=0.00
GA_38001	NM_152312	hypothetical protein FLJ35207 (FLJ35207)	4	1	0	0	5	es 12.01	p=0.02
GA_38023	NM_015846	methyl-CpG binding domain protein 1 (MBD1), transcript variant 1	7	0	1	0	8	es 21.02	p=0.00
GA_38029			4	1	0	0	5	es 12.01	p=0.02
GA_38084	NM_015658	DKFZP564C186 protein (DKFZP564C186)	13	5	3	5	26	es 3.00	p=0.01
GA_3818	NM_006833	COP9 subunit 6 (MOV34 homolog, 34 kD) (COPS6)	8	1	1	6	16	es 3.00	p=0.04
GA_38225	NM_007152	zinc finger protein 195 (ZNF195) $\otimes$	4	0	2	0	6	es 6.01	p=0.04
GA_38238	AL133439	mRNA full length insert cDNA clone EUROIMAGE 200978	4	0	2	0	6	es 6.01	p=0.04
GA_38243	BM920378	AGENCOURT_6709352 NIH_MGC_122cDNA clone IMAGE:5750332 5' sequence	5	2	1	1	9	es 3.75	p=0.05
GA_3826	NM_006875	pim-2 oncogene (PIM2)	5	0	1	0	6	es 15.02	p=0.00
GA_38266	NM_144504	junctional adhesion molecule 1 (JAM1), transcript variant 5	18	4	3	8	33	es 3.60	p=0.00
GA_38278	NM_019852	methyltransferase like 3 (METTL3)	8	0	4	3	15	es 3.43	p=0.02
GA_38283	NM_013411	adenylate kinase 2 (AK2), nuclear gene encoding mitochondrial protein, transcript variant AK2B	16	6	6	3	31	es 3.20	p=0.00
GA_38292	NM_005455	zinc finger protein 265 (ZNF265) $\otimes$	6	2	3	0	11	es 3.60	p=0.03
GA_38304	NM_002394	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 (SLC3A2)	4	0	1	0	5	es 12.01	p=0.02

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_38370	NM_024923	nucleoporin 210 (NUP210)	8	0	2	1	11	es 8.01	p=0.00
GA_38371	NM_018003	uveal autoantigen with coiled-coil domains and ankyrin repeats (UACA)	5	1	1	2	9	es 3.75	p=0.05
GA_38377	NM_033288	KRAB zinc finger protein KR18 (KR18)	5	2	1	0	8	es 5.01	p=0.03
GA_38426	NG_001332	T cell receptor alpha delta locus (TCRA/TCRD) on chromosome 14	7	1	2	0	10	es 7.01	p=0.00
GA_38431	NM_021238	TERA protein (TERA)	26	5	2	8	41	es 5.21	p=0.00
GA_38500	AB040903	KIAA1470 protein sequence	21	12	7	7	47	es 2.43	p=0.00
GA_3851	NM_006759	UDP-glucose pyrophosphorylase 2 (UGP2)	17	4	5	2	28	es 4.64	p=0.00
GA_38548	AB033107	KIAA1281 protein sequence	6	2	0	3	11	es 3.60	p=0.03
GA_3861	NM_006845	kinesin family member 2C (KIF2C)	9	1	4	1	15	es 4.51	p=0.00
GA_38627	AL831836	hypothetical protein sequence	5	1	1	2	9	es 3.75	p=0.05
GA_38635	NM_133370	KIAA1966 protein (KIAA1966)	9	4	4	2	19	es 2.70	p=0.03
GA_38666	BC000401	splicing factor 3b, subunit 2, 145kD sequence	16	9	9	6	40	es 2.00	p=0.04
GA_38677	NM_153280	ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing) (UBE1), transcript variant 2	44	41	10	14	109	es 2.03	p=0.00
GA_38691	NM_004550	NADH dehydrogenase (ubiquinone) Fe-S protein 2, 49kDa (NADH-coenzyme Q reductase) (NDUFS2)	9	1	2	6	18	es 3.00	p=0.02
GA_387	AB020648	KIAA0841 protein sequence	4	1	1	0	6	es 6.01	p=0.04

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Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_38786	NM_138769	mitochondrial Rho 2 (MIRO-2)	8	0	2	3	13	es 4.81	p=0.01
GA_38804	NM_018249	CDK5 regulatory subunit associated protein 2 (CDK5RAP2)	5	3	1	0	9	es 3.75	p=0.05
GA_38826	NM_133171	engulfment and cell motility 2 (ced-12 homolog, C. elegans) (ELMO2), transcript variant 1	4	1	0	1	6	es 6.01	p=0.04
GA_38854	NM_032228	hypothetical protein FLJ22728 (FLJ22728)	5	2	0	2	9	es 3.75	p=0.05
GA_38867	NM_018189	hypothetical protein FLJ10713 (FLJ10713)	34	2	6	1	43	es 11.35	p=0.00
GA_3897	NM_007015	chondromodulin I precursor (CHM-I)	4	0	1	0	5	es 12.01	p=0.02
GA_3898	NM_006892	DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B)	49	2	3	1	55	es 24.53	p=0.00
GA_3899	NM_144733	E1B-55kDa-associated protein 5 (E1B-AP5), transcript variant 2	23	16	6	7	52	es 2.38	p=0.00
GA_3938	NM_006925	splicing factor, arginine/serine-rich 5 (SFRS5)	29	4	24	6	63	es 2.56	p=0.00
GA_3984	NM_006114	translocase of outer mitochondrial membrane 40 homolog (yeast) (TOMM40)	7	1	2	2	12	es 4.20	p=0.01
GA_4038	NM_007223	putative G protein coupled receptor (GPR)	5	2	0	0	7	es 7.51	p=0.01
GA_4059	NM_007221	polyamine-modulated factor 1 (PMF1)	6	2	2	1	11	es 3.60	p=0.03
GA_4148	NM_003826	N-ethylmaleimide-sensitive factor attachment protein, gamma (NAPG)	4	1	0	1	6	es 6.01	p=0.04
GA_4176	NM_004448	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (ERBB2)	15	11	2	5	33	es 2.50	p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_4247	NM_001975	enolase 2, (gamma, neuronal) (ENO2)	5	0	2	0	7	es 7.51	p=0.01
GA_4251	NM_002528	nth endonuclease III-like 1 (E. coli) (NTHL1)	4	0	0	1	5	es 12.01	p=0.02
GA_4253	NM_004761	RAB2, member RAS oncogene family-like (RAB2L)	6	3	2	0	11	es 3.60	p=0.03
GA_4255	NM_006929	superkiller viralicidic activity 2-like (S. cerevisiae) (SKIV2L)	5	4	0	0	9	es 3.75	p=0.05
GA_4258	NM_080911	uracil-DNA glycosylase (UNG), nuclear gene encoding mitochondrial protein, transcript variant 2	9	3	6	0	18	es 3.00	p=0.02
GA_4263	NM_006247	protein phosphatase 5, catalytic subunit (PPP5C)	6	1	3	1	11	es 3.60	p=0.03
GA_4268	NM_003852	transcriptional intermediary factor 1 (TIF1)	13	4	4	1	22	es 4.34	p=0.00
GA_4295	NM_005255	cyclin G associated kinase (GAK)	6	3	2	0	11	es 3.60	p=0.03
GA_4302	NM_005054	RAN binding protein 2-like 1 (RANBP2L1), transcript variant 1	4	0	0	1	5	es 12.01	p=0.02
GA_4332	NM_019900	ATP-binding cassette, sub-family C (CFTR/MRP), member 1 (ABCC1), transcript variant 5	8	3	2	1	14	es 4.00	p=0.01
GA_4446	NM_002388	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae) (MCM3)	38	4	8	7	57	es 6.01	p=0.00
GA_4478	AK074826	cDNA FLJ90345 fis, clone NT2RP2002974, highly similar to HOMEBOX PROTEIN SIX5 sequence	4	0	0	0	4	es >4	p=0.00
GA_4551	NM_007375	TAR DNA binding protein (TARDBP)	17	11	4	5	37	es 2.55	p=0.01
GA_4568	NM_012100	aspartyl aminopeptidase (DNPEP)	8	1	1	1	11	es 8.01	p=0.00
GA_458	AF080158	IkB kinase-b sequence	4	0	0	0	4	es >4	p=0.00

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Geron ID	GenBank ID	Name	EST counts				Relative Expression			
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GA_4619	NM_012295	calcineurin binding protein 1 (CABIN1)	6	4	1	0	11	es 3.60	p=0.03	
GA_4659	NM_134434	RAD54B homolog (RAD54B), transcript variant 2	4	0	2	0	6	es 6.01	p=0.04	
GA_4689	NM_012470	transportin-SR (TRN-SR)	11	4	3	1	19	es 4.13	p=0.00	
GA_4693	NM_012256	zinc finger protein 212 (ZNF212)	⊗	5	0	1	2	8	es 5.01	p=0.03
GA_4694	NM_012482	zinc finger protein 281 (ZNF281)	⊗	4	0	0	0	4	es >4	p=0.00
GA_4788	NM_016263	Fzr1 protein (FZR1)		5	1	0	3	9	es 3.75	p=0.05
GA_4802	AB033092	KIAA1266 protein sequence		9	4	2	0	15	es 4.51	p=0.00
GA_4973	NM_015503	SH2-B homolog (SH2B)		5	2	1	1	9	es 3.75	p=0.05
GA_5037	AB037847	KIAA1426 protein sequence		6	2	3	0	11	es 3.60	p=0.03
GA_5052	NM_015705	hypothetical protein DJ1042K10.2 (DJ1042K10.2)		9	2	2	1	14	es 5.41	p=0.00
GA_5301	NM_145251	serine/threonine/tyrosine interacting protein (STYX)		4	0	0	0	4	es >4	p=0.00
GA_5391	NM_002968	sal-like 1 (Drosophila) (SALL1)		7	1	1	0	9	es 10.51	p=0.00
GA_5470	NM_002610	pyruvate dehydrogenase kinase, isoenzyme 1 (PDK1), nuclear gene encoding mitochondrial protein		4	0	1	1	6	es 6.01	p=0.04
GA_5475	NM_012280	FtsJ homolog 1 (E. coli) (FTSJ1)		6	0	1	0	7	es 18.02	p=0.00
GA_5493	NM_005415	solute carrier family 20 (phosphate transporter), member 1 (SLC20A1)		6	1	0	3	10	es 4.51	p=0.02

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
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GA_5504	NM_007318	presenilin 1 (Alzheimer disease 3) (PSEN1), transcript variant l-463	5	1	1	2	9	es 3.75	p=0.05
GA_5513	NM_014324	alpha-methylacyl-CoA racemase (AMACR)	4	0	1	0	5	es 12.01	p=0.02
GA_5534	NM_014316	calcium regulated heat stable protein 1, 24kDa (CARHSP1)	8	1	3	1	13	es 4.81	p=0.01
GA_5620	NM_014516	CCR4-NOT transcription complex, subunit 3 (CNOT3)	8	5	1	2	16	es 3.00	p=0.04
GA_5622	NM_014434	NADPH-dependent FMN and FAD containing oxidoreductase (NR1)	5	0	1	0	6	es 15.02	p=0.00
GA_5665	NM_014264	serine/threonine kinase 18 (STK18)	5	1	1	2	9	es 3.75	p=0.05
GA_5703	NM_134264	SOCS box-containing WD protein SWIP-1 (WSB1), transcript variant 3	44	29	9	12	94	es 2.64	p=0.00
GA_5729	NM_015456	cofactor of BRCA1 (COBRA1)	7	2	2	0	11	es 5.26	p=0.01
GA_5735	NM_015537	DKFZP586J1624 protein (DKFZP586J1624)	4	1	0	1	6	es 6.01	p=0.04
GA_5811	NM_014669	KIAA0095 gene product (KIAA0095)	10	3	4	0	17	es 4.29	p=0.00
GA_5829	NM_014773	KIAA0141 gene product (KIAA0141)	8	1	2	3	14	es 4.00	p=0.01
GA_5836	NM_014865	chromosome condensation-related SMC-associated protein 1 (KIAA0159)	12	5	4	2	23	es 3.28	p=0.01
GA_5906	NM_014675	KIAA0445 gene product (KIAA0445)	5	3	1	0	9	es 3.75	p=0.05
GA_5911	NM_014857	KIAA0471 gene product (KIAA0471)	4	0	0	2	6	es 6.01	p=0.04
GA_5954	NM_014871	KIAA0710 gene product (KIAA0710)	5	2	0	0	7	es 7.51	p=0.01

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression
			ES	EB	preHEP	preNeu Total	
GA_5961	NM_014828	chromosome 14 open reading frame 92 (C14orf92)	7	3	0	3	es 3.50 p=0.02
GA_5981	NM_014921	lectomedin-2 (KIAA0821)	11	5	0	1	es 5.51 p=0.00
GA_6007	NM_014962	BTB (POZ) domain containing 3 (BTBD3)	7	0	3	3	es 3.50 p=0.02
GA_6011	NM_014963	KIAA0963 protein (KIAA0963)	4	1	0	0	es 12.01 p=0.02
GA_6106	NM_015888	hook1 protein (HOOK1)	5	0	0	1	es 15.02 p=0.00
GA_6133	NM_016335	proline dehydrogenase (oxidase) 1 (PRODH), nuclear gene encoding mitochondrial protein	5	1	2	0	es 5.01 p=0.03
GA_6139	NM_016448	RA-regulated nuclear matrix-associated protein (RAMP)	6	1	2	0	es 6.01 p=0.01
GA_6232	NM_016223	protein kinase C and casein kinase substrate in neurons 3 (PACSLIN3)	5	1	1	1	es 5.01 p=0.03
GA_6271	NM_016518	pipecolic acid oxidase (PIPOX)	4	0	0	0	es >4 p=0.00
GA_6317	NM_015935	CGI-01 protein (CGI-01)	7	2	1	3	es 3.50 p=0.02
GA_638	AB024494	huntingtin interacting protein 3 sequence	4	0	2	0	es 6.01 p=0.04
GA_6438	NM_002889	retinoic acid receptor responder (tazarotene induced) 2 (RARRES2)	4	0	0	1	es 12.01 p=0.02
GA_6445	NM_017424	cat eye syndrome chromosome region, candidate 1 (CECR1)	10	2	2	4	es 3.75 p=0.01
GA_6460	NM_017415	kelch-like 3 (Drosophila) (KLHL3)	4	0	0	0	es >4 p=0.00
GA_6649	NM_148956	Williams Beuren syndrome chromosome region 20A (WBSR20A), transcript variant 1	4	0	0	0	es >4 p=0.00

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Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_6665	NM_018077	hypothetical protein FLJ10377 (FLJ10377)	7	0	2	3	12	es 4.20	p=0.01
GA_6669	NM_018085	importin 9 (FLJ10402)	12	0	3	3	18	es 6.01	p=0.00
GA_6673	NM_018093	hypothetical protein FLJ10439 (FLJ10439)	5	2	0	2	9	es 3.75	p=0.05
GA_6731	NM_018182	hypothetical protein FLJ10700 (FLJ10700)	7	0	2	1	10	es 7.01	p=0.00
GA_6742	NM_018198	hypothetical protein FLJ10737 (FLJ10737)	8	4	3	0	15	es 3.43	p=0.02
GA_6760	NM_018228	chromosome 14 open reading frame 115 (C14orf115)	13	1	0	0	14	es 39.05	p=0.00
GA_6806	NM_018303	homolog of yeast Sec5 (SEC5)	5	1	1	1	8	es 5.01	p=0.03
GA_6905	NM_017722	hypothetical protein FLJ20244 (FLJ20244)	4	1	0	1	6	es 6.01	p=0.04
GA_6957	NM_017815	chromosome 14 open reading frame 94 (C14orf94)	4	0	0	1	5	es 12.01	p=0.02
GA_6975	NM_017840	mitochondrial ribosomal protein L16 (MRPL16), nuclear gene encoding mitochondrial protein	6	0	2	2	10	es 4.51	p=0.02
GA_7078	NM_015148	PAS domain containing serine/threonine kinase (PASK)	5	0	0	0	5	es >4	p=0.00
GA_7155	NM_007098	clathrin, heavy polypeptide-like 1 (CLTCL1), transcript variant 2	4	0	1	0	5	es 12.01	p=0.02
GA_7158	NM_017489	telomeric repeat binding factor (NIMA-interacting) 1 (TERF1), transcript variant 1	14	3	2	3	22	es 5.26	p=0.00
GA_7170	NM_019013	hypothetical protein FLJ10156 (FLJ10156)	7	1	3	2	13	es 3.50	p=0.02
GA_7178	NM_019079	hypothetical protein FLJ10884 (FLJ10884)	34	2	4	1	41	es 14.59	p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_7334	NM_020347	leucine zipper transcription factor-like 1 (LZTFL1)	6	2	1	0	9	es 6.01 p=0.01
GA_7382	AB040878	KIAA1445 protein sequence	7	1	0	2	10	es 7.01 p=0.00
GA_7542			21	0	4	0	25	es 15.77 p=0.00
GA_7691	D42046	The ha3631 gene product is related to S.cerevisiae protein encoded in chromosome VIII. sequence	4	1	1	0	6	es 6.01 p=0.04
GA_8100	NM_054013	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isoenzyme B (MGAT4B), transcript variant 2	5	1	1	2	9	es 3.75 p=0.05
GA_8103	NM_144570	HN1 like (HN1L)	14	2	4	4	24	es 4.20 p=0.00
GA_8119	NM_012266	DnaJ (Hsp40) homolog, subfamily B, member 5 (DNAJB5)	4	1	0	1	6	es 6.01 p=0.04
GA_8152	AK095108	cDNA FLJ37789 fis, clone BRHIP3000081 sequence	6	2	1	0	9	es 6.01 p=0.01
GA_82	NM_015545	KIAA0632 protein (KIAA0632)	5	1	1	1	8	es 5.01 p=0.03
GA_8484	AK026658	cDNA: FLJ23005 fis, clone LNG00396, highly similar to AF055023clone 24723 mRNA sequence	4	0	0	0	4	es >4 p=0.00
GA_8559	NM_022497	mitochondrial ribosomal protein S25 (MRPS25), nuclear gene encoding mitochondrial protein	6	1	3	1	11	es 3.60 p=0.03
GA_8603	NM_007175	chromosome 8 open reading frame 2 (C8orf2)	7	3	1	1	12	es 4.20 p=0.01
GA_8667			4	0	0	0	4	es >4 p=0.00
GA_8686	Z24725	mitogen inducible gene mig-2 sequence	10	3	0	3	16	es 5.01 p=0.00

TABLE 5: EST Frequency of Genes that Down-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_8730	AK098833	cDNA FLJ25967 fis, clone CBR01929 sequence	10	3	2	0	15	es 6.01	p=0.00
GA_8803	NM_000533	proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated) (PLP1)	6	3	0	0	9	es 6.01	p=0.01
GA_8862	AK091593	cDNA FLJ34274 fis, clone FEBRA2003327 sequence	5	0	0	0	5	es >4	p=0.00
GA_9014									
GA_9162	AF311912	pancreas tumor-related protein sequence	6	0	1	1	8	es 9.01	p=0.00
GA_9163	NM_138639	BCL2-like 12 (proline rich) (BCL2L12), transcript variant 1	7	1	0	4	12	es 4.20	p=0.01
GA_9167	AF308602	NOTCH 1 sequence	8	1	3	0	12	es 6.01	p=0.00
GA_9183	NM_007129	Zic family member 2 (odd-paired homolog, Drosophila) (ZIC2)	6	2	1	0	9	es 6.01	p=0.01
GA_9257	NM_005088	DNA segment on chromosome X and Y (unique) 155 expressed sequence (DXYS155E)	8	1	1	0	10	es 12.01	p=0.00
GA_9338	NM_020436	similar to SALL1 (sal (Drosophila)-like (LOC57167)	4	1	0	1	6	es 6.01	p=0.04
GA_9365	NM_021078	GCN5 general control of amino-acid synthesis 5-like 2 (yeast) (GCN5L2)	11	2	3	0	16	es 6.61	p=0.00
GA_9384	NM_020997	left-right determination, factor B (LEFTB)	7	1	2	1	11	es 5.26	p=0.01
GA_9388	NM_021643	GS3955 protein (GS3955)	4	0	1	0	5	es 12.01	p=0.02
GA_9488	NM_007372	RNA helicase-related protein (RNAHP)	7	1	0	2	10	es 7.01	p=0.00
			12	7	1	6	26	es 2.57	p=0.02

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Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_9571	NM_022130	golgi phosphoprotein 3 (coat-protein) (GOLPH3)	6	2	2	1	11	es 3.60 p=0.03
GA_9593	NM_022372	G protein beta subunit-like (GBL)	6	0	1	1	8	es 9.01 p=0.00
GA_96	NM_012297	Ras-GTPase activating protein SH3 domain-binding protein 2 (KIAA0660)	19	9	6	8	42	es 2.48 p=0.00
GA_9664	NM_015339	activity-dependent neuroprotector (ADNP)	7	1	2	2	12	es 4.20 p=0.01
GA_9688	NM_022767	hypothetical protein FLJ12484 (FLJ12484)	14	3	1	3	21	es 6.01 p=0.00
GA_9697	NM_022778	hypothetical protein DKFZp434L0117 (DKFZP434L0117)	6	2	1	0	9	es 6.01 p=0.01
GA_9784	NM_021873	cell division cycle 25B (CDC25B), transcript variant 3	5	2	0	1	8	es 5.01 p=0.03
GA_9829	BM454622	AGENCOURT_6406365 NIH_MGC_92cDNA clone IMAGE:5583082 5' sequence	6	1	1	0	8	es 9.01 p=0.00
GA_9952	BC003542	Unknown (protein for IMAGE:3611719) sequence	6	0	1	0	7	es 18.02 p=0.00
GA_9996	NM_005911	methionine adenosyltransferase II, alpha (MAT2A)	27	8	9	14	58	es 2.62 p=0.00

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_10484	AK056774	unnamed protein product sequence	4	153	17	34	208	es 0.06 p=0.00
GA_10493	NM_023009	MARCKS-like protein (MLP)	6	7	15	32	60	es 0.33 p=0.01
GA_1071	NM_001641	APEX nuclease (multifunctional DNA repair enzyme) 1 (APEX1), transcript variant 1	5	13	15	12	45	es 0.38 p=0.04
GA_11334	NM_032272	homolog of yeast MAF1 (MAF1)	0	4	7	1	12	es 0.00 p=0.05
GA_11407	NM_015070	KIAA0853 protein (KIAA0853)	0	2	2	8	12	es 0.00 p=0.05
GA_12217	BC009917	Unknown (protein for MGC:2764) sequence	0	7	3	5	15	es 0.00 p=0.03
GA_1222	NM_001901	connective tissue growth factor (CTGF)	2	26	4	14	46	es 0.14 p=0.00
GA_12727	NM_004926	zinc finger protein 36, C3H type-like 1 (ZFP36L1)	3	8	12	22	45	es 0.21 p=0.00
GA_1336	NM_002024	fragile X mental retardation 1 (FMR1)	0	3	4	7	14	es 0.00 p=0.03
GA_1353	NM_002051	GATA binding protein 3 (GATA3)	0	2	8	2	12	es 0.00 p=0.05
GA_1403	NM_001530	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) (HIF1A)	4	22	5	8	39	es 0.34 p=0.04
GA_1432	NM_002166	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein (ID2)	1	3	17	4	25	es 0.13 p=0.01
GA_1476	NM_002276	keratin 19 (KRT19)	1	26	14	38	79	es 0.04 p=0.00
GA_1545	NM_002512	non-metastatic cells 2, protein (NM23B) expressed in (NME2), nuclear gene encoding mitochondrial protein	3	6	7	16	32	es 0.31 p=0.04

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_1556	NM_003633	ectodermal-neural cortex (with BTB-like domain) (ENC1)	1	5	2	28	36	es 0.09	p=0.00
GA_1735	NM_002806	proteasome (prosome, macropain) 26S subunit, ATPase, 6 (PSMC6)	1	7	7	8	23	es 0.14	p=0.03
GA_1736	NM_002814	proteasome (prosome, macropain) 26S subunit, non-ATPase, 10 (PSMD10)	0	4	10	5	19	es 0.00	p=0.01
GA_1841	NM_000979	ribosomal protein L18 (RPL18)	4	6	36	35	81	es 0.16	p=0.00
GA_1843	NM_000982	ribosomal protein L21 (RPL21)	1	7	48	42	98	es 0.03	p=0.00
GA_1850	BC020169	clone IMAGE:3543815, partial cds	0	2	8	11	21	es 0.00	p=0.00
GA_1857	NM_000999	ribosomal protein L38 (RPL38)	1	2	12	10	25	es 0.13	p=0.01
GA_1866	NM_002950	ribophorin I (RPN1)	3	12	10	14	39	es 0.25	p=0.01
GA_1886	NM_001009	ribosomal protein S5 (RPS5)	8	14	46	30	98	es 0.27	p=0.00
GA_1977	NM_003134	signal recognition particle 14kDa (homologous Alu RNA binding protein) (SRP14)	1	4	18	12	35	es 0.09	p=0.00
GA_2014	NM_003564	transgelin 2 (TAGLN2)	5	31	8	28	72	es 0.22	p=0.00
GA_2039	NM_003246	thrombospondin 1 (THBS1)	0	3	2	7	12	es 0.00	p=0.05
GA_23018	NM_005336	high density lipoprotein binding protein; vigilin sequence	11	37	17	21	86	es 0.44	p=0.01
GA_23176			2	18	3	7	30	es 0.21	p=0.02
GA_23180	AB009010	polyubiquitin UbC, complete cds	7	16	23	26	72	es 0.32	p=0.00

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Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_23653	NM_003289	tropomyosin 2 (beta) (TPM2)	2	14	7	8	31	es 0.21	p=0.01
GA_23969			0	1	181	20	202	es 0.00	p=0.00
GA_24037			0	1	6	5	12	es 0.00	p=0.05
GA_2524	NM_004415	desmoplakin (DPI, DPII) (DSP)	3	14	5	23	45	es 0.21	p=0.00
GA_2597	NM_138610	H2A histone family, member Y (H2AFY), transcript variant 3	1	5	5	14	25	es 0.13	p=0.01
GA_2627	NM_004905	anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent phospholipase A2) (AOP2)	3	6	11	17	37	es 0.27	p=0.01
GA_2702	NM_000942	peptidylprolyl isomerase B (cyclophilin B) (PPIB)	5	6	7	26	44	es 0.39	p=0.04
GA_2752	NM_004175	small nuclear ribonucleoprotein D3 polypeptide 18kDa (SNRPD3)	0	1	9	4	14	es 0.00	p=0.03
GA_2782	NM_004786	thioredoxin-like, 32kDa (TXNL)	0	4	1	10	15	es 0.00	p=0.03
GA_2808	NM_001154	annexin A5 (ANXA5)	2	14	4	11	31	es 0.21	p=0.01
GA_2968	BC007090	histidine triad nucleotide-binding protein, clone MGC:14708 IMAGE:4250172, complete cds	0	1	11	9	21	es 0.00	p=0.00
GA_3016	NM_001873	carboxypeptidase E (CPE)	1	8	4	9	22	es 0.14	p=0.02
GA_3026	NM_005722	ARP2 actin-related protein 2 homolog (yeast) (ACTR2)	6	19	7	19	51	es 0.40	p=0.03
GA_3033	NM_005717	actin related protein 2/3 complex, subunit 5, 16kDa (ARPC5)	3	10	8	19	40	es 0.24	p=0.01

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_3036	NM_152862	actin related protein 2/3 complex, subunit 2, 34kDa (ARPC2), transcript variant 1	1	9	3	7	20	es 0.16	p=0.04
GA_3126	NM_005620	S100 calcium binding protein A11 (calgizzarin) (S100A11)	0	1	7	37	45	es 0.00	p=0.00
GA_3132	NM_005625	syndecan binding protein (syntenin) (SDCBP)	1	3	10	10	24	es 0.13	p=0.02
GA_3260	NM_006004	ubiquinol-cytochrome c reductase hinge protein (UQCRH)	1	4	12	5	22	es 0.14	p=0.02
GA_3283	NM_004484	glypican 3 (GPC3)	1	6	7	12	26	es 0.12	p=0.01
GA_3294	NM_006476	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g (ATP5L)	0	1	3	11	15	es 0.00	p=0.03
GA_33625	NM_058179	phosphoserine aminotransferase (PSA), transcript variant 1	2	8	5	14	29	es 0.22	p=0.03
GA_33660	BF528488	602043661F1 NCL_CGAP_Bm67cDNA clone IMAGE:4181462 5' sequence	0	7	7	2	16	es 0.00	p=0.02
GA_33787	AL832673	mRNA; cDNA DKFZp313B1017 (from clone DKFZp313B1017) sequence	0	3	4	6	13	es 0.00	p=0.05
GA_3403	NM_006142	stratifin (SFN)	0	2	1	14	17	es 0.00	p=0.01
GA_3431	NM_006294	ubiquinol-cytochrome c reductase binding protein (UQCRB)	0	2	9	7	18	es 0.00	p=0.01
GA_3435	NM_006472	thioredoxin interacting protein (TXNIP)	4	14	16	11	45	es 0.29	p=0.01
GA_34569	NM_003299	tumor rejection antigen (gp96) 1 (TRA1)	3	9	27	20	59	es 0.16	p=0.00
GA_34776	NM_002273	keratin 8 (KRT8)	9	71	144	156	380	es 0.07	p=0.00

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

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Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_34912	NM_006367	adenylyl cyclase-associated protein (CAP)	9	24	10	31	74	es 0.42	p=0.01
GA_34930	NM_000700	annexin A1 (ANXA1)	2	12	3	15	32	es 0.20	p=0.01
GA_35086	NM_002128	high-mobility group box 1 (HMGB1)	1	3	8	8	20	es 0.16	p=0.04
GA_35179	NM_001402	eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)	16	29	43	63	151	es 0.36	p=0.00
GA_3530	NM_002539	ornithine decarboxylase 1 (ODC1)	1	10	8	9	28	es 0.11	p=0.01
GA_35369	NM_003374	voltage-dependent anion channel 1 (VDAC1)	1	5	6	10	22	es 0.14	p=0.02
GA_35434	NM_006094	deleted in liver cancer 1 (DLC1)	0	8	1	5	14	es 0.00	p=0.03
GA_35463	NM_024298	leukocyte receptor cluster (LRC) member 4 (LENG4)	0	4	9	8	21	es 0.00	p=0.00
GA_3560	NM_003079	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1 (SMARCE1)	2	5	11	11	29	es 0.22	p=0.03
GA_35641	BC029424	similar to weakly similar to glutathione peroxidase 2 sequence	1	11	5	3	20	es 0.16	p=0.04
GA_35978	NM_006830	ubiquinol-cytochrome c reductase (6.4kD) subunit (UQCR)	0	1	4	7	12	es 0.00	p=0.05
GA_3617	NM_000391	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease) (CLN2)	1	4	15	2	22	es 0.14	p=0.02
GA_36322	NM_001554	cysteine-rich, angiogenic inducer, 61 (CYR61)	0	3	3	7	13	es 0.00	p=0.05
GA_36460	NM_001300	core promoter element binding protein (COPEB)	0	6	2	7	15	es 0.00	p=0.03

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
GA_3652	NM_005556	keratin 7 (KRT7)	0	9	1	14	24	es 0.00	p=0.00
GA_36638	NM_002954	ribosomal protein S27a (RPS27A)	3	5	37	35	80	es 0.12	p=0.00
GA_36721	NM_005134	protein phosphatase 4, regulatory subunit 1 (PPP4R1)	0	8	2	6	16	es 0.00	p=0.02
GA_36891	NM_001019	ribosomal protein S15a (RPS15A)	0	2	50	32	84	es 0.00	p=0.00
GA_36932	NM_015338	KIAA0978 protein (KIAA0978)	0	5	3	5	13	es 0.00	p=0.05
GA_3707	NM_003816	a disintegrin and metalloproteinase domain 9 (meltrin gamma) (ADAM9)	0	8	1	3	12	es 0.00	p=0.05
GA_37238	NM_021019	myosin, light polypeptide 6, alkali, smooth muscle and non-muscle (MYL6), transcript variant 1	0	2	2	12	16	es 0.00	p=0.02
GA_37377	NM_000516	GNAS complex locus (GNAS), transcript variant 1	12	16	27	38	93	es 0.44	p=0.01
GA_37494	NM_001305	claudin 4 (CLDN4)	1	2	10	12	25	es 0.13	p=0.01
GA_37508	NM_000994	ribosomal protein L32 (RPL32)	2	6	26	35	69	es 0.09	p=0.00
GA_37557	NM_152437	hypothetical protein DKFZp761B128 (DKFZp761B128)	1	7	13	3	24	es 0.13	p=0.02
GA_37660	NM_001749	calpain, small subunit 1 (CAPNS1)	4	7	11	20	42	es 0.32	p=0.02
GA_37689	AK022962	cdNA FLJ12900 fis, clone NT2RP2004321 sequence	0	4	6	2	12	es 0.00	p=0.05
GA_37776	NM_000366	tropomyosin 1 (alpha) (TPM1)	24	46	37	74	181	es 0.46	p=0.00
GA_3782	NM_003968	ubiquitin-activating enzyme E1C (UBA3 homolog, yeast) (UBE1C)	0	1	5	6	12	es 0.00	p=0.05

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts				Relative Expression		
			ES	EB	preHEP	preNeu		Total	
GA_3789	NM_006818	ALL1-fused gene from chromosome 1q (AF1Q)	0	17	1	11	29	es 0.00	p=0.00
GA_38037	NM_033480	F-box only protein 9 (FBXO9), transcript variant 2	0	4	4	4	12	es 0.00	p=0.05
GA_3812	NM_006854	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2 (KDELFR2)	3	12	5	17	37	es 0.27	p=0.01
GA_38124	NM_000269	non-metastatic cells 1, protein (NM23A) expressed in (NME1)	1	2	8	13	24	es 0.13	p=0.02
GA_38191	NM_000224	keratin 18 (KRT18)	8	46	50	119	223	es 0.11	p=0.00
GA_38341	NM_006931	solute carrier family 2 (facilitated glucose transporter), member 3 (SLC2A3)	28	49	45	85	207	es 0.47	p=0.00
GA_38503	NM_000612	insulin-like growth factor 2 (somatomedin A) (IGF2)	0	17	4	21	42	es 0.00	p=0.00
GA_38528	NM_012062	dynamitin 1-like (DNM1L), transcript variant 1	0	5	4	3	12	es 0.00	p=0.05
GA_38545	NM_005801	putative translation initiation factor (SUI1)	1	14	15	19	49	es 0.06	p=0.00
GA_38563	NM_021005	nuclear receptor subfamily 2, group F, member 2 (NR2F2)	0	9	8	9	26	es 0.00	p=0.00
GA_3857	NM_006644	heat shock 105kD (HSP105B)	1	11	3	7	22	es 0.14	p=0.02
GA_38570	NM_033150	collagen, type II, alpha 1 (primary osteoarthritis, spondyloepiphyseal dysplasia, congenital) (COL2A1), transcript variant 2	0	15	31	5	51	es 0.00	p=0.00
GA_38790	NM_001743	calmodulin 2 (phosphorylase kinase, delta) (CALM2)	15	23	36	37	111	es 0.47	p=0.00
GA_38817	NM_013341	hypothetical protein PTD004 (PTD004)	0	4	5	3	12	es 0.00	p=0.05

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_38830	NM_006013	ribosomal protein L10 (RPL10)	12	13	71	81	177	es 0.22 p=0.00
GA_3892	NM_006888	calmodulin 1 (phosphorylase kinase, delta) (CALM1)	1	3	11	9	24	es 0.13 p=0.02
GA_3973	NM_144497	A kinase (PRKA) anchor protein (gravin) 12 (AKAP12), transcript variant 2	0	17	1	20	38	es 0.00 p=0.00
GA_3977	NM_005139	annexin A3 (ANXA3)	0	3	4	10	17	es 0.00 p=0.01
GA_4045	NM_003897	immediate early response 3 (IER3), transcript variant short	1	14	2	4	21	es 0.15 p=0.04
GA_4132	NM_002305	lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	0	5	2	7	14	es 0.00 p=0.03
GA_4182	NM_001202	bone morphogenetic protein 4 (BMP4), transcript variant 1	0	7	6	4	17	es 0.00 p=0.01
GA_4395	NM_003145	signal sequence receptor, beta (translocon-associated protein beta) (SSR2)	6	17	12	14	49	es 0.42 p=0.05
GA_4418	NM_004800	transmembrane 9 superfamily member 2 (TM9SF2)	0	7	2	8	17	es 0.00 p=0.01
GA_4615	NM_012286	MORF-related gene X (MRGX)	10	22	16	23	71	es 0.49 p=0.04
GA_4640	NM_012342	putative transmembrane protein (NIMA)	1	8	3	10	22	es 0.14 p=0.02
GA_4914	NM_016282	adenylate kinase 3 like 1 (AK3L1)	0	2	6	4	12	es 0.00 p=0.05
GA_5243	NM_139207	nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1	7	19	28	25	79	es 0.29 p=0.00
GA_5387	NM_002047	glycyl-tRNA synthetase (GARS)	8	9	34	34	85	es 0.31 p=0.00
GA_5557	NM_014211	gamma-aminobutyric acid (GABA) A receptor, pi (GABRP)	1	3	4	13	21	es 0.15 p=0.04

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression	
			ES	EB	preHEP	preNeu	Total		
		(GABRP)							
GA_5730	NM_015641	testis derived transcript (3 LIM domains) (TES), transcript variant 1	0	2	2	9	13	es 0.00	p=0.05
GA_5992	NM_014899	Rho-related BTB domain containing 3 (RHOBTB3)	0	10	7	13	30	es 0.00	p=0.00
GA_6118	NM_016403	hypothetical protein HSPC148 (HSPC148)	0	2	7	3	12	es 0.00	p=0.05
GA_6136	NM_016368	myo-inositol 1-phosphate synthase A1 (ISYNA1)	1	7	5	16	29	es 0.11	p=0.00
GA_6165	NM_015853	ORF (LOC51035)	1	5	9	5	20	es 0.16	p=0.04
GA_6219	NM_016139	16.7Kd protein (LOC51142)	1	5	13	14	33	es 0.09	p=0.00
GA_6381	NM_016641	membrane interacting protein of RGS16 (MIR16)	0	2	3	7	12	es 0.00	p=0.05
GA_6388	NM_016145	PTD008 protein (PTD008)	0	1	2	10	13	es 0.00	p=0.05
GA_6437	NM_016732	RNA binding protein (autoantigenic, hnRNP-associated with lethal yellow) (RALY), transcript variant 1	2	6	7	12	27	es 0.24	p=0.04
GA_6481	NM_014380	nerve growth factor receptor (TNFRSF16) associated protein 1 (NGFRAP1)	1	4	8	17	30	es 0.10	p=0.00
GA_7280	NM_020199	HTGN29 protein (HTGN29)	0	6	2	6	14	es 0.00	p=0.03
GA_7286	NM_172316	Meis1, myeloid ecotropic viral integration site 1 homolog 2 (mouse) (MEIS2), transcript variant h	0	4	2	10	16	es 0.00	p=0.02
GA_749	BC015794	Unknown (protein for MGC:8837) sequence	0	4	4	9	17	es 0.00	p=0.01
GA_7520	NM_003486	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 (SLC7A5)	2	20	3	20	45	es 0.14	p=0.00

TABLE 6: EST Frequency of Genes that Up-regulate upon Differentiation

Geron ID	GenBank ID	Name	EST counts					Relative Expression
			ES	EB	preHEP	preNeu	Total	
GA_7635	NM_170746	selenoprotein H (SELH)	0	1	10	2	13	es 0.00 p=0.05
GA_8275	NM_012203	glyoxylate reductase/hydroxypyruvate reductase (GRHPR)	0	3	2	12	17	es 0.00 p=0.01
GA_8627	NM_006868	RAB31, member RAS oncogene family (RAB31)	0	5	1	7	13	es 0.00 p=0.05
GA_8674	NM_000598	insulin-like growth factor binding protein 3 (IGFBP3)	1	15	4	3	23	es 0.14 p=0.03
GA_8980	NM_005347	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5)	10	29	15	30	84	es 0.41 p=0.01
GA_9152	NM_005324	H3 histone, family 3B (H3.3B) (H3F3B)	20	26	57	49	152	es 0.46 p=0.00
GA_9196	NM_000404	galactosidase, beta 1 (GLB1), transcript variant 179423	0	6	10	7	23	es 0.00 p=0.00
GA_9251	NM_004373	cytochrome c oxidase subunit VIa polypeptide 1 (COX6A1), nuclear gene encoding mitochondrial protein	0	3	7	8	18	es 0.00 p=0.01
GA_9266	NM_021104	ribosomal protein L41 (RPL41)	6	9	70	75	160	es 0.12 p=0.00
GA_9649	NM_014604	Tax interaction protein 1 (TIP-1)	0	8	5	5	18	es 0.00 p=0.01
GA_9734	NM_022908	hypothetical protein FLJ12442 (FLJ12442)	0	3	2	14	19	es 0.00 p=0.01

Example 3: Microarray analysis for other differentially expressed genes

In another series of experiments, the level of gene expression was tested at the mRNA level in microarrays.

Genes were selected from the non-redundant set of gene assemblies from the four cDNA libraries described in Example 1, based on their novelty and possible interest as markers. An additional 7,000 sequence-verified clones were obtained from Research Genetics (Huntsville AL) and incorporated into an array with a control set of ~200 known housekeeping genes. Each clone was grown overnight in 96-well format and DNA purified using the Qiagen 96-well DNA kit. The DNA templates were PCR amplified in 100 µL reactions. PCR product was then purified using the ArrayIt™ PCR Purification Kit (Telechem, Sunnyvale CA) according to manufacturer instructions. Product was dried down, resuspended in 50% DMSO and ArrayIt™ Microprinting solution (Telechem, Sunnyvale CA) and arrayed onto GAPSTM amino silane coated slides (Corning Inc., Acton MA) using a GMS 417 Arrayer (Affymetrix, Santa Clara, CA). After printing, slides were humidified and snap heated, baked at 80° for 4 h, then blocked with succinic anhydride.

Total RNA from undifferentiated ES cells, embryoid body cells (EB), retinoic acid treated (preNeu), and DMSO treated (PreHep) cells S, EB, RA-treated, and DMSO-treated cells (10 µg, 15 µg, and 20 µg for sensitivity) was then reverse transcriptase labeled with Cy3 or Cy5 fluorophores, and competitively hybridized to the microarrays overnight at 42°C in 50% formamide and Sigma hybridization buffer. Undifferentiated ES RNA was directly and indirectly compared with RNA from all other cell types. Experiments were repeated at least 5 times each, and dye reversed. Stratagene Universal Human Reference RNA (Cat. #740000) was used as the indirect comparator. Arrays were washed repeatedly and scanned using a GenePix™ 4000A microarray scanner (Axon Instruments, Fremont CA).

Image processing, data extraction and preliminary quality control were performed using GenePix™ Pro 3.0.6 (Axon Instruments). Quality control calculations involved quantifying overall signal intensities, statistical means and medians of pixel intensities and spot morphologies. Extracted data was further analyzed based on statistical algorithms of signal-to-noise, sensitivity range, and reproducibility. Data was then loaded into the GeneSpring™ database and analysis program. Of particular interest were genes that showed reproducible expression differences of 2-fold in either direction, especially when the change occurred upon differentiation to all three differentiated cell types.

The following table lists genes that were identified as being downregulated or upregulated in their expression level upon differentiation into EB, preHEP, or preNEU cells. EST counts are provided from the data generated in the previous example.

TABLE 7: Microarray Analysis — Genes that Decrease Expression upon Differentiation

Geron ID	GenBank ID	Name	Fold Change		EST Counts			
			RA	DMSO	ES	EB	preHep	preNeu
GA_1674	NM_002701	POU domain, class 5, transcription factor 1 (POU5F1)	-3.61	-10.68	24	1	2	0
GA_9384	NM_020997	left-right determination, factor B (LEFTB)	-4.88	-5.48	4	0	1	0
GA_37788	NM_133631	roundabout, axon guidance receptor, homolog 1	-7.93	-2.9	7	4	1	0
GA_12173	NM_021912	gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3)	-3.37	-2.16	4	0	0	0
GA_37606	NM_019012	phosphoinositol 3-phosphate-binding protein-2 (PEPP2)	-2.96	-9.99	4	2	0	0
GA_1470	NM_003740	potassium channel, subfamily K, member 5 (KCNK5)	-2.93	-2.47	4	0	0	1
GA_2937	NM_005207	v-crkl sarcoma virus CT10 oncogene homolog (avian)-like (CRKL)	-2.29	-3.78	6	1	0	0
GA_10513	NM_033209	Thy-1 co-transcribed (LOC94105)	-2.21	-3.39	7	2	2	1
GA_36957	NM_024642	N-acetylgalactosaminyltransferase 12 (GalNAc-T12) (GALNT12)	-3.24	-5.05	4	0	1	1
GA_36420	NM_001064	transketolase (Wernicke-Korsakoff syndrome) (TKT)	-2.25	-2.28	14	17	11	17
GA_1677	NM_003712	phosphatidic acid phosphatase type 2C (PPAP2C)	-2.46	-2.71	1	0	0	0
GA_36793	NM_152295	threonyl-tRNA synthetase (TARS)	-2.18	-3.5	8	4	1	6
GA_7151	NM_017488	adducin 2 (beta) (ADD2), transcript variant beta-4	-4.21	-2.03	4	2	2	0
GA_12053	NM_001986	ets variant gene 4 (E1A enhancer binding protein, E1AF) (ETV4)	-2.76	-2.04	0	1	0	4
GA_1798	NM_000964	retinoic acid receptor, alpha (RARA)	-2.76	-3.3	3	2	0	0
GA_5617	NM_014502	nuclear matrix protein NMP200 related to splicing factor PRP19 (NMP200)	-2.19	-2.33	5	3	4	2
GA_2753	NM_000582	secreted phosphoprotein 1 (osteopontin) (SPP1)	-3.78	-3.32	3	6	2	39
GA_7151	NM_017486	adducin 2 (beta) (ADD2), transcript variant beta-6a	-3.34	-2.13	4	2	2	0
GA_36775	NM_000918	procollagen-proline, thyroid hormone binding protein p55) (P4HB)	-2.01	-2.65	12	28	10	22
GA_1086	NM_133436	asparagine synthetase (ASNS), transcript variant 1	-2.27	-2.53	6	5	3	13
GA_2928	NM_005163	v-akt murine thymoma viral oncogene homolog 1 (AKT1)	-2.79	-3.45	2	10	2	5
GA_33799	NM_003250	thyroid hormone receptor (THRA)	-4.28	-4.44	0	2	0	1
GA_37861	NM_021784	forkhead box A2 (FOXA2), transcript variant 1	-3.56	-2.99	2	0	0	0

TABLE 7: Microarray Analysis — Genes that Decrease Expression upon Differentiation

Geron ID	GenBank ID	Name	Fold Change		EST Counts			
			RA	DMSO	ES	EB	preHep	preNeu
GA_34109	NM_002026	fibronectin 1 (FN1), transcript variant 1	-2.91	-2.01	17	166	5	27
GA_38641	NM_004309	Rho GDP dissociation inhibitor (GDI) alpha (ARHGDIA)	-2.72	-2.35	7	8	9	14
GA_33829	NM_002081	glypican 1 (GPC1)	-2.61	-2.32	3	9	4	1
GA_5549	NM_014600	EH-domain containing 3 (EHD3)	-2.39	-2.81	1	5	1	1
GA_9269	NM_021074	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa (NDUFV2)	-2.26	-2.01	0	0	9	6
GA_2934	NM_005180	B lymphoma Mo-MLV insertion region (mouse) (BMI1)	-2.11	-3.24	1	2	0	1
GA_3522	NM_002415	macrophage migration inhibitory factor (glycosylation-inhibiting factor) (MIF)	-2.04	-2.05	4	2	8	9
GA_2465	NM_004364	CCAAT/enhancer binding protein (C/EBP), alpha (CEBPA)	-2.79	-4	0	1	0	0
GA_36793	NM_152295	threonyl-tRNA synthetase (TARS)	-5.34	-2.98	8	4	1	6
GA_9259	NM_005539	inositol polyphosphate-5-phosphatase, 40kDa (INPP5A)	-4.37	-6.54	1	0	0	2
GA_2232	NM_001348	death-associated protein kinase 3 (DAPK3)	-2.9	-3.56	3	3	1	2
GA_37240	NM_007029	stathmin-like 2 (STMN2)	-4.37	-2.37	0	4	0	1
GA_4617	NM_012289	Kelch-like ECH-associated protein 1 (KEAP1)	-11.88	-2.59	2	4	2	2
GA_38021	NM_002111	huntingtin (Huntington disease) (HD)	-10.84	-2.16	1	5	0	2
GA_9227	NM_001552	insulin-like growth factor binding protein 4 (IGFBP4)	-6.13	-3.06	5	4	0	2
GA_267	NM_007041	arginyltransferase 1 (ATE1)	-3.03	-3.22	1	1	0	2
GA_38392	NM_006597	heat shock 70kDa protein 8 (HSPA8), transcript variant 1	-8.8	-2.7	39	20	48	62
GA_1829	NM_002936	ribonuclease H1 (RNASEH1)	-2.81	-2.11	1	0	1	2
GA_9228	NM_001664	ras homolog gene family, member A (ARHA)	-3.21	-2.48	11	18	8	17
GA_1495	NM_002347	lymphocyte antigen 6 complex, locus H (LY6H)	-2.33	-2.57	0	0	0	1
GA_3840	NM_006749	solute carrier family 20 (phosphate transporter), member 2 (SLC20A2)	-5.4	-2.83	0	1	1	3
GA_1045	NM_001105	activin A receptor, type I (ACVR1)	-2.7	-2.37	0	3	1	3
GA_36361	NM_020636	zinc finger protein 275 (ZNF275)	-4.09	-2.07	0	0	0	3

TABLE 7: Microarray Analysis — Genes that Decrease Expression upon Differentiation

Geron ID	GenBank ID	Name	Fold Change		EST Counts			
			RA	DMSO	ES	EB	preHep	preNeu
GA_2445	NM_004337	chromosome 8 open reading frame 1 (C8orf1)	-3.02	-2.2	1	0	0	0
GA_4652	NM_012228	pilin-like transcription factor (PILB)	-2.73	-2.46	0	0	1	0
GA_10567	NM_025195	phosphoprotein regulated by mitogenic pathways (C8FW)	-4.74	-3.64	0	2	0	1
GA_9258	NM_005393	plexin B3 (PLXNB3)	-3.56	-3.04	0	2	0	0
GA_35992	NM_001402	eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)	-5.55	-2.22	419	467	454	428
GA_33537	NM_133259	leucine-rich PPR-motif containing (LRPPRC)	-2.47	-3.41	8	7	5	3
GA_6367	NM_016354	solute carrier family 21 (organic anion transporter), member 12 (SLC21A12)	-2.08	-3.26	0	0	0	1
GA_667	AB028976	mRNA for KIAA1053 protein, partial cds	-7.55	-3.52	0	2	0	2
	BQ023180	NCI_CGAP_P16 cDNA clone UI-1-BB1p-aui-g-05-0-UI 3' sequence	-2.96	-2.1				
	AA419281	Soares ovary tumor NbHOT cDNA clone IMAGE:755641 3' sequence	-3.36	-2.59				
	NM_006604	ret finger protein-like 3 (RFPL3)	-2.69	-2.5				
	NM_012155	echinoderm microtubule associated protein like 2 (EML2)	-9.82	-6.65				
	NM_000160	glucagon receptor (GCGR)	-3.94	-2.18				
	NM_003181	T, brachyury homolog (mouse) (T)	-9.15	-2.11				
	NM_014620	homeo box C4 (HOXC4), transcript variant 1	-9.54	-2.1				
	NM_005583	lymphoblastic leukemia derived sequence 1 (LYL1)	-4.36	-2.79				
	NM_014310	RASD family, member 2 (RASD2)	-2.72	-3.13				
	NM_012467	tryptase gamma 1 (TPSG1)	-2.63	-2.55				
	NM_000539	rhodopsin (opsin 2, rod pigment) (retinitis pigmentosa 4, autosomal dominant) (RHO)	-4.84	-5.53				
	NM_021076	neurofilament, heavy polypeptide (200kD) (NEFH)	-2.03	-2.41				
	NM_012407	protein kinase C, alpha binding protein (PRKCABP)	-5.44	-2.56				
	NM_000201	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor (ICAM1)	-2.18	-2.06				

TABLE 8: Microarray Analysis — Genes that Increase Expression upon Differentiation

Geron ID	GenBank ID	Name	Fold Change		EST Counts			
			RA	DMSO	ES	EB	preHep	preNeu
GA_1055	NM_001134	alpha-fetoprotein (AFP)	8.02	5.07	0	4	0	0
GA_1055	NM_001134	alpha-fetoprotein (AFP)	6.45	3.71	0	4	0	0
GA_1055	NM_001134	alpha-fetoprotein (AFP)	2.58	2.67	0	4	0	0
GA_1213	NM_001884	cartilage linking protein 1 (CRTL1)	4.57	8.71	3	1	17	3
GA_1476	NM_002276	keratin 19 (KRT19)	2.09	5.21	1	26	14	38
GA_8674	NM_000598	insulin-like growth factor binding protein 3 (IGFBP3)	3.16	3.59	1	15	4	3
GA_3283	NM_004484	glypican 3 (GPC3)	2.6	3.29	1	6	7	12
GA_37735	NM_058178	neuronal pentraxin receptor (NPTXR)	3.77	4.04	1	0	0	1
GA_1280	NM_001957	endothelin receptor type A(EDNRA)	3.05	6.37	2	2	1	0
GA_37308	NM_003068	snail homolog 2 (Drosophila) (SNAI2)	2.24	4.68	4	3	0	0
GA_5909	NM_014851	KIAA0469 gene product	2.77	2.03	3	3	0	1
GA_23450	XM_027313	ATP synthase mitochondrial F1 complex assembly factor 1 (ATPAF1),	2.48	3.55	3	1	1	1
GA_7286	NM_020119	likely ortholog of rat zinc-finger antiviral protein (ZAP)	2.5	3.55	1	0	0	0

Example 4: Specificity of expression confirmed by real-time PCR

To verify the expression patterns of particular genes of interest at the mRNA level, extracts of undifferentiated hES cells and their differentiated progeny were assayed by real-time PCR. Cells were cultured for 1 week with 0.5% dimethyl sulfoxide (DMSO) or 500 nM retinoic acid (RA). The samples were amplified using sequence-specific primers, and the rate of amplification was correlated with the expression level of each gene in the cell population.

Taqman™ RT-PCR was performed under the following conditions: 1 × RT Master Mix (ABI), 300 nM for each primer, and 80 nM of probe, and 10 pg to 100 ng of total RNA in nuclease-free water. The reaction was conducted under default RT-PCR conditions of 48°C hold for 30 min, 95°C hold for 10 min, and 40 cycles of 95°C at 15 sec and 60°C hold for 1 min. RNA was isolated by a guanidinium isothiocyanate method (RNAeasy™ kit, Qiagen) according to manufacturer's instructions, and subsequently DNase treated (DNAfree™ kit, Ambion). Gene-specific primers and probes were designed by PrimerExpress™ software (Ver. 1.5, ABI). Probe oligonucleotides were synthesized with the fluorescent indicators 6-carboxyfluorescein (FAM) and 6-carboxy-tetramethylrhodamine (TAMRA) at the

5' and 3' ends, respectively. Relative quantitation of gene expression between multiple samples was achieved by normalization against endogenous 18S ribosomal RNA (primer and probe from ABI) using the $\Delta\Delta C_T$ method of quantitation (ABI). Fold change in expression level was calculated as $2^{-\Delta\Delta C_T}$.

The table below shows the results of this analysis. Since the cells have been cultured in RA and DMSO for a short period, they are at the early stages of differentiation, and the difference in expression level is less dramatic than it would be after further differentiation. Of particular interest for following or modulating the differentiation process are markers that show modified expression within the first week of differentiation by more than 2-fold (*), 5-fold (**), 10-fold (***), or 100-fold (****).

TABLE 9: Quantitative RT-PCR analysis of gene expression in hESC differentiation

Geron ID	GenBank ID	Name	Fold Change	
			RA	DMSO
A. GA_10902	NM_024504	Pr domain containing 14 (PRDM14) **	-1.9	-8.3
GA_11893	NM_032805	Hypothetical protein FLJ14549 ***	-2.3	-10.0
GA_12318	NM_032447	Fibrillin3		
GA_1322	NM_000142	Fibroblast growth factor receptor 3 precursor (FGFR-3) *	1.5	2.3
GA_1329	NM_002015	Forkhead box o1a (foxo1a) *	-1.6	-2.9
GA_1470	NM_003740	Potassium channel subfamily k member 5 (TASK-2)	-1.6	1.0
GA_1674	NM_002701	Octamer-binding transcription factor 3a (OCT-3A) (OCT-4) **	-3.7	-7.7
GA_2024	NM_003212	Teratocarcinoma-derived growth factor 1 (CRIPTO) ***	-4.0	-12.5
GA_2149	NM_003413	Zic family member 3 (ZIC3) **	-1.7	-5.3
GA_2334	NM_000216	Kallmann syndrome 1 sequence (KAL1) *	-1.1	-2.5
GA_23552	BC027972	Glypican-2 (cerebroglycan)	-1.5	-1.2
GA_2356	NM_002851	Protein tyrosine phosphatase, receptor-type, z polypeptide 1 (PTPRZ1) *	-1.7	-3.3
GA_2367	NM_003923	Forkhead box h1 (FOXH1) **	-1.8	-5.6
GA_2436	NM_004329	Bone morphogenetic protein receptor, type Ia (BMPRI1A) (ALK-3) *	-2.4	-2.4
GA_2442	NM_004335	Bone marrow stromal antigen 2 (BST-2)	1.1	-1.9
GA_2945	NM_005232	Ephrin type-a receptor 1 (EPHA1)	-1.3	-1.9
GA_2962	NM_005314	Gastrin-releasing peptide receptor (GRP-R) **	-6.3	-9.1
GA_2988	NM_005397	Podocalyxin-like (PODXL) *	-2.6	-4.3
GA_3337	NM_006159	Nell2 (NEL-like protein 2)	-1.3	-1.3
GA_3559	NM_005629	Solute carrier family 6, member 8 (SLC6A8)	-1.1	-1.1

TABLE 9: Quantitative RT-PCR analysis of gene expression in hESC differentiation

Geron ID	GenBank ID	Name	Fold Change	
			RA	DMSO
GA_420	X98834	Zinc finger protein, HSAL2 *	-1.4	-2.8
GA_5391	NM_002968	Sal-like 1 (SALL1),	1.4	-1.3
GA_6402	NM_016089	Krab-zinc finger protein SZF1-1 *	-1.8	-3.1
GA_9167	AF308602	Notch 1 (N1)	1.3	1.0
GA_9183	AF193855	Zinc finger protein of cerebellum ZIC2 *	1.0	-2.9
GA_9443	NM_004426	Early development regulator 1 (polyhomeotic 1 homolog) (EDR1) **	-1.8	-5.6
B. GA_9384	NM_020997	Left-right determination, factor b (LEFTB) **	-16.7	-25.0
GA_12173	BC010641	Gamma-aminobutyric acid (GABA) A receptor, beta 3 **	-2.8	-5.6
GA_10513	NM_033209	Thy-1 co-transcribed ***	-12.5	-11.1
GA_1831	NM_002941	Roundabout, axon guidance receptor, homolog 1 (ROBO1),	1.1	1.0
GA_2753	NM_000582	Secreted phosphoprotein 1 (osteopontin) ***	-3.8	-10.0
GA_32919	NM_133259	130 kDa leucine-rich protein (LRP 130)	-1.9	-1.9
GA_28290	AK055829	FLJ31267 (acetylglucosaminyltransferase-like protein) *	-2.3	-4.5
C. GA_28053	T24677	EST ****	< -100*	< -100*
GA_26303	NM_138815	Hypothetical protein BC018070 ***	-3.2	-10.0
GA_2028	NM_003219	Telomerase reverse transcriptase (TERT) *	-2.1	-2.3

Example 5: Selection of markers for monitoring ES cell differentiation

Genes that undergo up- or down-regulation in expression levels during differentiation are of interest for a variety of different commercial applications, as described earlier. This experiment provides an example in which certain genes were selected as a means to monitor the ability of culture conditions to maintain the undifferentiated cell phenotype — and hence, the pluripotent differentiation capability of the cells.

Particular genes were chosen from those identified as having differential expression patterns, because they are known or suspected of producing a protein gene product that is expressed at the cell surface, or is secreted. These attributes are helpful, because they allow the condition of the cells to be monitored easily either by antibody staining of the cell surface, or by immunoassay of the culture supernatant. Genes were chosen from the EST database (Groups 1), microarray analysis (Group 2), and other sources (Group 3).

TABLE 10: Additional Genes analyzed by real-time PCR

	Name	GenBank or ID No.
Group 1	Bone marrow stromal antigen	NM_004335
	Podocalyxin-like	NM_005397
	Rat GPC/ glypican-2 (cerebroglycan)	TA_5416486
	Potassium channel subfamily k member 5 (TASK-2)	NM_003740
	Notch 1 protein	AF308602
	Teratocarcinoma-derived growth factor 1 (Cripto)	NM_003212
	Nel 1 like / NELL2 (Nel-like protein 2)	NM_006159
	Gastrin releasing peptide receptor	NM_005314
	Bone morphogenetic protein receptor	NM_004329
	ABCG2- ABC transporter	AY017168
	Solute carrier family 6, member 8 (SLC6A8)	NM_005629
	hTERT	NM_003219
	Oct 3/4 octamer-binding transcription factor 3a (oct-3a) (oct-4)	NM_002701
Group 2	Left-right determination factor b (LEFTB)	NM_020997
	Secreted phosphoprotein 1 (osteopontin)	NM_000582
	Gamma-aminobutyric acid (GABA) A receptor, beta 3	NM_021912
	Roundabout, axon guidance receptor, homologue 1 (ROBO1),	NM_002941
	Glucagon receptor	NM_00160
	Leucine-rich PPR-motif hum 130 kDa hum130leu 130kd Leu	M92439
	Thy-1 co-transcribed	NM_033209
	Solute carrier family 21	NM_016354
	LY6H lymphocyte antigen 6 complex locus H	NM_002347
	Plexin (PLXNB3)	NM_005393
	ICAM	NM_000201
Group 3	Rhodopsin	NM_000539
	Kallmann syndrome 1 sequence (KAL1)	NM_000216
	Armadillo repeat protein deleted in velo-cardio-facial syndrome (ARVCF)	NM_001670
	Ephrin type-a receptor 1 (EPHA1)	NM_005232

Figure 1 shows the decrease in expression of the genes in Group I (Upper Panel) and Group II (Lower Panel) in H9 hES cells after culturing for 7 days with RA or DM. Gene expression of rhodopsin and ICAM was below the limit of detection in differentiated cells. KAL1 and EPHA1 were not tested.

Besides hTERT and Oct 3/4, three other genes were selected as characteristic of the undifferentiated hES cell phenotype. They were Teratocarcinoma-derived growth factor (Cripto), Podocalyxin-like (PODXL), and gastrin-releasing peptide receptor (GRPR).

Figure 2 compares the level of expression of these five genes in hES cells with fully differentiated cells: BJ fibroblasts, BJ fibroblasts transfected to express hTERT (BJ-5TA), and 293 (human embryonic kidney) cells. The level of all markers shown was at least 10-fold higher, and potentially more than 10^2 , 10^3 , 10^4 , 10^5 , or 10^6 -fold higher in pluripotent stem cells than fully differentiated cells. All five markers retained a detectable level of expression in differentiated cultures of hESC. It is not clear if there is lower level of expression of these markers in differentiated cells, or if the detectable expression derived from the undifferentiated cells in the population. The one exception observed in this experiment was the hTERT transgene, expressed at an elevated level as expected in the BJ-5TA cells.

High-level expression of Cripto, GRPR and PODXL in undifferentiated hES cells reveals interesting aspects of the biology of these cells. Cripto has been implicated in normal mammalian development and tumor growth. Cripto encodes a glycosylphosphoinositol anchored protein that contains an EGF repeat and a cysteine rich motif, which makes it a member of the EGF-CFC family. It has been demonstrated that Cripto serves as a co receptor for Nodal, which is essential for mesoderm and endoderm formation in vertebrate development (Yeo et al., Molecular Cell 7:949, 2001). The finding that Cripto is expressed preferentially on undifferentiated hESC suggests that Nodal is an important signaling molecule for stem cells, perhaps to promote survival and/or proliferation.

PODXL encodes for transmembrane sialoprotein that is physically linked to the cytoskeleton. PODXL is suspected to act as an inhibitor of cell-cell adhesion and has been implicated in the embryonic development of the kidney podocyte. The anti-adhesion properties of PODXL when expressed on undifferentiated hESC may be an important feature related to stem cell migration.

The receptor for gastrin releasing peptide (GRP) is a G-protein coupled receptor that mediates numerous biological effects of Bombesin-like peptides, including regulation of gut acid secretion and satiety. A critical role has also been established for GRP and GRPR in control growth of cultured cells and normal mammalian development. GRP and GRPR may be oncofetal antigens that act as morphogens in normal development and cancer.

Example 6: Use of cell markers to modify ES cell culture conditions

This example illustrates the utility of the differentially expressed genes identified according to this invention in the evaluation of culture environments suitable for maintaining pluripotent stem cells.

Figure 3 show results of an experiment in which hES cells of the H1 line were maintained for multiple passages in different media. Medium conditioned with feeder cells provides factors effective to allow hES cells to proliferate in culture without differentiating. However, culturing in unconditioned medium leads to loss of the undifferentiated phenotype, with an increasing percentage of the cells showing decreased expression of CD9 (a marker for endothelial cells, fibroblasts, and certain progenitor cells), and the classic hES cell marker SSEA-4.

Figure 4 illustrates the sensitivity of hTERT, Oct 3/4, Cripto, GRP receptor, and podocalyxin-like protein (measured by real-time PCR assay) as a means of determining the degree of differentiation of the cells. After 4 passages in unconditioned X-VIVO™ 10 medium containing 8 ng/mL bFGF, all 5 markers show expression that has been downregulated by about 10-fold. After 8 passages, expression has decreased by 10^2 , 10^3 , or 10^4 -fold.

Figure 5 shows results of an experiment in which the hES cell line H1 was grown on different feeder cell lines: mEF = mouse embryonic fibroblasts; hMSC = human mesenchymal stem cells; UtSMC = human uterine smooth muscle cells; WI-38 = an established line of human lung fibroblasts. As monitored by RT-PCR assay of Cripto, Oct 3/4, and hTERT, at least under the conditions used in this experiment, the hMSC are better substitutes for mEF feeders than the other cell lines tested.

Figure 6 shows results of an experiment in which different media were tested for their ability to promote growth of hES cells without differentiation. Expression of Podocalyxin-like protein, Cripto, GFP Receptor, and hTERT were measured by RT-PCR. The test media were not preconditioned, but supplemented with the growth factors as follows:

TABLE 11: Growth Conditions Tested for Marker Expression

Standard conditions:	DMEM preconditioned with mEF+ bFGF (8 ng/mL)
Condition 3	X-VIVO™ 10 + bFGF (8 ng/mL)
Condition 4	X-VIVO™ 10 + bFGF (40 ng/mL)
Condition 5	X-VIVO™ 10 + bFGF (40 ng/mL) + stem cell factor (SCF, 15 ng/mL)
Condition 6	X-VIVO™ 10 + bFGF (40 ng/mL) + Flt3 ligand (75 ng/mL)
Condition 7	X-VIVO™ 10 + bFGF (40 ng/mL) + LIF (100 ng/mL)
Condition 8	QBSF™-60 + bFGF (40ng/mL)

The results show that the markers selected to monitor the undifferentiated phenotype showed similar changes in each of these culture conditions. By all criteria, X-VIVO 10™ supplemented according to Condition 6 was found to be suitable for culturing hES cells without having to be preconditioned. As shown on the right side, when cells were put back into standard conditioned medium after 8 passages in the test conditions, expression of all four markers returned essentially to original levels. This shows that alterations in expression profiles in media Conditions 4 to 8 are temporary and reversible — consistent with the cells retaining full pluripotency.

Example 7: Measuring undifferentiated cell markers by flow cytometry

Cells from the undifferentiated hES cell line H1 were grown in mEF conditioned medium in Matrigel® coated 6-well plates. Cells were harvested using 3.0 mL of 0.5 mM EDTA and resuspended in PBS containing 5% fetal calf serum and 0.05% NaN₃ at a concentration of 5×10^6 cells/mL. For SSEA-4

and TRA1-60 staining, 1 μ g of antibody (Chemicon International) was used. Cells were incubated for a period of 30 min on ice followed by one wash with 2.0 mL of PBS-FCS buffer. Cell pellets were resuspended in 100 μ L of fluorochrome conjugated secondary antibody. For intracellular Oct-4 staining, the cells were fixed with 2% PFA (final concentration) for 15 min at room temperature. After one wash, cells were resuspended in a permeabilization buffer (PBS-FCS plus 90% cold methanol) followed by 15 min in ice, washed again, and then resuspended the cell pellet in blocking solution (20% goat serum in permeabilization buffer). 0.5×10^6 or 1.0×10^6 permeabilized cells were stained with 1 μ g of anti-Oct-4 antibody (Santa Cruz Biotechnology) in 10 μ L of blocking solution, incubated on ice for 30 min. After rewashing, the cells were stained with labeled secondary antibody.

Figure 7 shows that SSEA-4, TRA 1-60 and Oct-4 markers were all strongly expressed on undifferentiated cells under these conditions. Solid areas in each panel indicate background staining observed with the respective isotype-matched controls. In fact, greater than 85% of hES cells expressed all three markers.

Example 8: Measuring differentiated cells using stromal markers

The extent of differentiation can be determined by detecting or measuring markers for undifferentiated cells, in combination with markers for differentiated cells of the type expected in early differentiation cultures — either by antibody staining, or by PCR amplification (Taqman™), or by a combination of techniques.

In this example, screening of useful stromal cell markers was done by immunocytochemistry of hES cells cultured in XVIVO 10™ with bFGF, or medium conditioned using mouse embryonic fibroblasts. Antibodies were obtained from commercial sources as follows:

TABLE 12: Primary Antibody for Measuring Differentiated Cells

Marker	Vendor	Catalog No.
STRO-1	RnD Systems	MAB 1038
Human Thymus Stroma	BD Pharmingen	555825
CD44	BD Pharmingen	550988
CD90	BD Pharmingen	555593
CD105 (Endoglin)	Chemicon	MAB2152
CD106 (VCAM-1)	BD Pharmingen	555645
Vimentin	Sigma	V 5255

Figure 8 shows the results of the immunocytochemical analysis. CD44, STRO-1 and Vimentin stain stromal-like cells in the hES cell populations cultured with mEF conditioned medium.

Example 8: Sensitivity of the assay for undifferentiated cells

Real-time PCR assays were performed using mixtures of undifferentiated hES cells and BJ fibroblasts, to determine the sensitivity of the assay for the presence of differentiated cells.

Freshly harvested cells were combined to a total of 2×10^6 cells in 10% increments of each cell type. Total RNA was isolated (Roche isolation kit), and then treated with DNase 1 to remove potential DNA contaminants. (Ambion kit). Amplification mixtures were made up in QRT-PCR master mix buffer (P/N 4309169) to a final volume of 25 μ L at a concentration of 10 μ M forward primer, 10 μ M reverse primer, 10 μ M probe, and ~100 ng RNA. Data analysis was performed using the comparative Ct method using 18S rRNA endogenous control. (Other suitable housekeeping genes for standardization can be used instead, such as acidic ribosomal protein, β -actin, cyclophilin, G3P dehydrogenase, or β 2-microglobulin).

Figure 9 shows the relative change of gene expression measured in mixtures of differentiated (BJ) and undifferentiated hES cells, compared with undifferentiated hES cells alone. These five markers are able to rank 10% changes in the proportion of undifferentiated cells.

SEQUENCE DATA

Sequences Referred to in this Disclosure

SEQ. ID NO:	Designation	Reference
	hTERT mRNA sequence	GenBank Accession NM_003129
	hTERT protein sequence	GenBank Accession NM_003129
	Oct 3/4 mRNA sequence	GenBank Accession NM_002701
	Oct 3/4 protein sequence	GenBank Accession NM_002701
	Cripto mRNA sequence	GenBank Accession NM_003212
	Cripto protein sequence	GenBank Accession NM_003212
	podocalyxin-like protein mRNA sequence	GenBank Accession NM_005397
	podocalyxin-like protein amino acid sequence	GenBank Accession NM_005397
	GRP receptor mRNA sequence	GenBank Accession NM_005314
	GRP receptor proteins sequence	GenBank Accession NM_005314
	Primers & probes for real-time PCR assay	This disclosure
	Human telomeric repeats	U.S. Patent 5,583,016
1	Geron sequence designation GA_12064	This disclosure
2	Geron sequence designation GA_23176	This disclosure
3	Geron sequence designation GA_23468	This disclosure
4	Geron sequence designation GA_23476	This disclosure
5	Geron sequence designation GA_23484	This disclosure
6	Geron sequence designation GA_23485	This disclosure
7	Geron sequence designation GA_23486	This disclosure
8	Geron sequence designation GA_23487	This disclosure
9	Geron sequence designation GA_23488	This disclosure
10	Geron sequence designation GA_23489	This disclosure
11	Geron sequence designation GA_23490	This disclosure
12	Geron sequence designation GA_23514	This disclosure
13	Geron sequence designation GA_23515	This disclosure
14	Geron sequence designation GA_23525	This disclosure
15	Geron sequence designation GA_23572	This disclosure
16	Geron sequence designation GA_23577	This disclosure
17	Geron sequence designation GA_23579	This disclosure

Sequences Referred to in this Disclosure

SEQ. ID NO:	Designation	Reference
18	Geron sequence designation GA_23585	This disclosure
19	Geron sequence designation GA_23596	This disclosure
20	Geron sequence designation GA_23615	This disclosure
21	Geron sequence designation GA_23634	This disclosure
22	Geron sequence designation GA_23673	This disclosure
23	Geron sequence designation GA_23683	This disclosure
24	Geron sequence designation GA_23969	This disclosure
25	Geron sequence designation GA_24037	This disclosure
26	Geron sequence designation GA_32842	This disclosure
27	Geron sequence designation GA_32860	This disclosure
28	Geron sequence designation GA_32895	This disclosure
29	Geron sequence designation GA_32913	This disclosure
30	Geron sequence designation GA_32917	This disclosure
31	Geron sequence designation GA_32926	This disclosure
32	Geron sequence designation GA_32947	This disclosure
33	Geron sequence designation GA_32979	This disclosure
34	Geron sequence designation GA_32985	This disclosure
35	Geron sequence designation GA_35405	This disclosure
36	Geron sequence designation GA_38029	This disclosure
37	Geron sequence designation GA_7542	This disclosure
38	Geron sequence designation GA_8667	This disclosure
39	Geron sequence designation GA_9014	This disclosure

LOCUS TERT 4015 bp mRNA linear PRI 31-OCT-2000
 DEFINITION Homo sapiens telomerase reverse transcriptase (TERT), mRNA.
 ACCESSION NM_003219
 AUTHORS Nakamura,T.M., Morin,G.B., Chapman,K.B., Weinrich,S.L.,
 Andrews,W.H., Lingner,J., Harley,C.B. and Cech,T.R.
 TITLE Telomerase catalytic subunit homologs from fission yeast and human
 JOURNAL Science 277 (5328), 955-959 (1997)
 CDS 56..3454

LOCUS POU5F1 1158 bp mRNA linear PRI 31-OCT-2000
 DEFINITION Homo sapiens POU domain, class 5, transcription factor 1 (POU5F1),
 mRNA.
 ACCESSION NM_002701
 AUTHORS Takeda,J., Seino,S. and Bell,G.I.
 TITLE Human Oct3 gene family: cDNA sequences, alternative splicing, gene
 organization, chromosomal location, and expression at low levels in
 adult tissues
 JOURNAL Nucleic Acids Res. 20 (17), 4613-4620 (1992)
 CDS 102..899

LOCUS TDGF1 2033 bp mRNA linear PRI 05-NOV-2002
 DEFINITION Homo sapiens teratocarcinoma-derived growth factor 1 (TDGF1), mRNA.
 ACCESSION NM_003212
 AUTHORS Dono,R., Montuori,N., Rocchi,M., De Ponti-Zilli,L., Ciccodicola,A.
 and Persico,M.G.
 TITLE Isolation and characterization of the CRIPTO autosomal gene and its
 X-linked related sequence
 JOURNAL Am. J. Hum. Genet. 49 (3), 555-565 (1991)
 CDS 248..814

LOCUS PODXL 5869 bp mRNA linear PRI 01-NOV-2000
 DEFINITION Homo sapiens podocalyxin-like (PODXL), mRNA.
 ACCESSION NM_005397
 AUTHORS Kershaw,D.B., Beck,S.G., Wharram,B.L., Wiggins,J.E., Goyal,M.,
 Thomas,P.E. and Wiggins,R.C.
 TITLE Molecular cloning and characterization of human podocalyxin-like
 protein. Orthologous relationship to rabbit PCLP1 and rat
 podocalyxin
 JOURNAL J. Biol. Chem. 272 (25), 15708-15714 (1997)
 CDS 251..1837

LOCUS GRPR 1726 bp mRNA linear PRI 05-NOV-2002
 DEFINITION Homo sapiens gastrin-releasing peptide receptor (GRPR), mRNA.
 ACCESSION NM_005314
 AUTHORS Xiao,D., Wang,J., Hampton,L.L. and Weber,H.C.
 TITLE The human gastrin-releasing peptide receptor gene structure, its
 tissue expression and promoter
 JOURNAL Gene 264 (1), 95-103 (2001)
 CDS 399..1553

* * * * *

*The subject matter provided in this disclosure can be modified as a matter of routine optimization,
 without departing from the spirit of the invention, or the scope of the appended claims.*

CLAIMS

1. A method for assessing a culture of undifferentiated primate pluripotent stem (pPS) cells or their progeny, comprising detecting or measuring expression of three or more of the markers in any of Tables 5 to 9.
2. The method of claim 1, comprising measuring expression of three or more of the markers in Tables 2, 7, and 9(C), and correlating the expression measured with the presence of undifferentiated embryonic stem (ES) cells in the culture.
3. The method of claim 1, comprising measuring expression of three or more of the markers in Tables 3 and 8, and correlating the expression measured with the presence of differentiated cells in the culture.
4. The method of claim 1, comprising detecting or measuring expression of one or more of the following markers: bone marrow stromal antigen; Podocalyxin-like; Rat GPC/ glypican-2 (cerebroglycan); Potassium channel subfamily k member 5 (TASK-2); Notch 1 protein; Teratocarcinoma-derived growth factor 1 (Cripto); Nel 1 like / NELL2 (Nel-like protein 2); Gastrin releasing peptide receptor; Bone morphogenetic protein; ABCG2- ABC transporter; Solute carrier family 6, member 8 (SLC6A8); hTERT; Oct 3/4 Octamer-binding transcription factor 3a (Oct-3a) (Oct-4); Left-right determination factor b (LEFT); Secreted phosphoprotein 1 (osteopontin); Gamma-aminobutyric acid (GABA) A receptor, beta 3; Roundabout, axon guidance receptor, homologue 1 (ROBO1),; Glucagon receptor; Leucine-rich ppr-motif hum 130 kDa hum130leu 130kd leu; Thy-1 co-transcribed; Solute carrier family 21; LY6H lymphocyte antigen 6 complex locus H; Plexin (PLXNB3); Armadillo repeat protein deleted in velo-cardio-facial syndrome; and Ephrin type-a receptor 1 (EPHA1).
5. The method of claim 1, comprising detecting or measuring expression of three or more of the markers in any of Tables 2, 7, and 9(C), and two or more of the markers in any of Tables 3 and 8
6. The method of claim 1 further comprising detecting or measuring expression of hTERT and/or Oct 3/4.
7. A method for assessing a culture of undifferentiated human embryonic stem (hES) cells or their progeny, comprising detecting or measuring two or more markers selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), and human telomerase reverse transcriptase (hTERT).
8. The method of claim 7, further comprising detecting or measuring one or more markers selected from Oct 3/4, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81.

9. The method of claim 7, comprising detecting or measuring Cripto, hTERT, and Oct 3/4.
10. A method for assessing a culture of undifferentiated human embryonic stem (hES) cells or their progeny, comprising detecting or measuring two or more markers preferentially expressed in undifferentiated hES cells, and one or more markers expressed preferentially after differentiation of the hES cells.
11. The method of claim 10, wherein at least one of the markers preferentially expressed in undifferentiated hES cells is selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), and human telomerase reverse transcriptase (hTERT).
12. The method of claim 11, wherein at least one of the markers preferentially expressed in undifferentiated hES cells is selected from Oct 3/4, SSEA-4, Tra-1-60 and Tra-1-81.
13. The method of claim 10, comprising detecting or measuring hTERT, Oct 3/4, and a marker selected from Cripto, SSEA-4, Tra-1-60 and Tra-1-81.
14. The method of claim 10, wherein at least one of the markers expressed preferentially after differentiation of the hES cells is a stromal cell markers.
15. The method of claim 14, wherein the stromal cell marker is selected from CD44, CD105 (endoglin), CD106 (VCAM-1), CD90 (Thy-1), STRO-1, Vimentin, and Human Thymus Stroma.
16. The method of claim 10, wherein expression of hTERT, Oct 3/4, Cripto, GRP receptor, PODXL, CD44, CD105, CD106, or CD90 is detected or measured at the mRNA level by PCR amplification.
17. The method of claim 10, wherein expression of SSEA-4, Tra-1-60, Tra-1-81, Cripto, Oct 3/4, CD44, CD105, CD106, CD90, STRO-1, Vimentin, or Human Thymus Stroma is detected or measured at the antigen expression level by antibody assay.
18. A kit for assessing a culture of pPS cells according to claim 1, comprising polynucleotide probes and/or primers for specifically amplifying a transcript for three or more markers in any of Tables 5 to 9, accompanied by written instructions for assessing the pPS cells according to the expression of said markers measured using the probes or primers in the kit.
19. A kit for assessing a culture of pPS cells according to claim 1, comprising an antibody specific for the gene product of three or more markers in any of Tables 5 to 9, accompanied by written instructions for assessing the pPS cells according to the expression of said markers measured using the antibody in the kit.

20. A kit for assessing a culture of undifferentiated human embryonic stem (hES) cells or their progeny according to claim 10, comprising antibody or PCR amplification primers specific for three or more markers, of which at least two are expressed preferentially in undifferentiated hES cells, and at least one is expressed preferentially in stromal cells.
21. The kit of claim 20, comprising antibody or PCR amplification primers specific for at least two markers selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), human telomerase reverse transcriptase (hTERT) Oct 3/4, SSEA-4, Tra-1-60, Tra-1-81, CD44, CD105 (endoglin), CD106 (VCAM-1), CD90 (Thy-1), STRO-1, Vimentin, and Human Thymus Stroma.
22. Use of antibody or PCR amplification primers specific for three or more markers selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), human telomerase reverse transcriptase (hTERT) Oct 3/4, SSEA-4, Tra-1-60, Tra-1-81, CD44, CD105 (endoglin), CD106 (VCAM-1), CD90 (Thy-1), STRO-1, Vimentin, and Human Thymus Stroma, for assessing a culture of undifferentiated human embryonic stem (hES) cells or their progeny.
23. The method of claim 1, which is a method for quantifying the proportion of undifferentiated pPS cells or differentiated cells in the culture.
24. The method of claim 1, which is a method for assessing the ability of a culture system or component thereof to maintain pPS cells in an undifferentiated state.
25. The method of claim 24, which is a method of assessing the ability of a soluble factor to maintain pPS cells in an undifferentiated state.
26. The method of claim 24, which is a method of assessing the ability of a culture medium to maintain pPS cells in an undifferentiated state.
27. The method of claim 24, which is a method of assessing the ability of a preparation of feeder cells to maintain pPS cells in an undifferentiated state.
28. The method of claim 1, which is a method for assessing the ability of a culture system or component thereof to cause differentiation of pPS cells into a culture of lineage-restricted precursor cells and/or terminally differentiated cells.
29. The method of claim 1, which is a method for assessing the suitability of a pPS cell culture for preparing cells for human administration.
30. The method of claim 1, wherein the level of the markers of undifferentiated hES cell markers is determined to be at least 100-fold higher than in BJ fibroblasts.

31. A method for assessing the growth characteristics of a cell population, comprising detecting or measuring expression of three or more of the markers in any of Tables 5 to 9.
32. A method for assessing the growth characteristics of a cell population,, comprising detecting or measuring two or more markers selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), and human telomerase reverse transcriptase (hTERT).
33. The method of claim 32, wherein the cell population has been obtained by culturing cells from a human blastocyst.
34. The method of claim 32, which is a method for determining whether the cell population is pluripotent.
35. The method of claim 31, wherein the cell population has been obtained from a human patient suspected of having a clinical condition related to abnormal cell growth.
36. The method of claim 31, which is a method for assessing whether the patient has a malignancy.
37. A method for maintaining pPS cells in a pluripotent state, comprising causing them to express one of the following markers at a higher level.
 - Forkhead box O1A (FOXO1A); Zic family member 3 (ZIC3); Hypothetical protein FLJ20582; Forkhead box H1 (FOXH1); Zinc finger protein, Hsa12; KRAB-zinc finger protein SZF1-1; and Zinc finger protein of cerebellum ZIC2.
38. The method of claim 37, wherein the cells are caused to express the marker by genetically altering it with a gene that encodes the marker.
39. A method for causing pPS cells to differentiate into a particular tissue type, comprising causing them to express one of the following markers at an altered level.
 - Forkhead box O1A (FOXO1A); Zic family member 3 (ZIC3); Hypothetical protein FLJ20582; Forkhead box H1 (FOXH1); Zinc finger protein, Hsa12; KRAB-zinc finger protein SZF1-1; Zinc finger protein of cerebellum ZIC2; and Coup transcription factor 2 (COUP-TF2).
40. The method of claim 39, wherein the cells are caused to express the marker by genetically altering it with a gene that encodes the marker, or with an antisense nucleic acid that binds to mRNA encoding the marker.
41. A method for causing an encoding sequence to be preferentially expressed in undifferentiated pPS cells, comprising genetically altering pPS cells with the encoding sequence under control of a promoter for one of the markers listed in any of Tables 2, 7, and 9(C).

42. The method of claim 41, which is a method for selecting undifferentiated cells, and the encoding sequence is a reporter gene (such as a gene that causes the cells to emit fluorescence), or a positive selection marker (such as a drug resistance gene).
43. The method of claim 41, which is a method for depleting undifferentiated cells from a population of differentiated cells, and the encoding sequence is a negative selection marker (such as a gene that activates apoptosis or converts a prodrug into a compound that is lethal to the cell).
44. A method for causing an encoding sequence to be preferentially expressed in differentiated cells, comprising genetically altering the pPS cells with the encoding sequence under control of a promoter for one of the markers listed in Table 3 or Table 8.
45. The method of claim 44, which is a method for selecting differentiated cells, and the encoding sequence is a reporter gene (such as a gene that causes the cells to emit fluorescence), or a positive selection marker (such as a drug resistance gene).
46. The method of claim 44, which is a method for depleting differentiated cells from a population of undifferentiated cells, and the encoding sequence is a negative selection marker (such as a gene that activates apoptosis or converts a prodrug into a compound that is lethal to the cell).
47. A method for sorting differentiated cells from less differentiated cells, comprising separating cells expressing a surface marker in any of Tables 5 to 9 from cells not expressing the marker.
48. The method of claim 47, wherein the cells are sorted using an antibody or lectin that binds the marker or product thereof on the cell surface.
49. A method for causing pPS cells to proliferate without differentiation, comprising culturing them in a culture system assessed according to the method of claim 7.
50. A method for causing pPS cells to proliferate without differentiation, comprising culturing them with human mesenchymal stem cells.

51. A method for identifying genes that are up- or down-regulated during differentiation of pPS cells, comprising:
- a) sequencing transcripts in an expression library from undifferentiated pPS cells;
 - b) sequencing transcripts in one or more expression libraries from one or more cell types that have differentiated from the same line of pPS cells;
 - c) determining the frequency of transcripts from each gene sequenced in each of the libraries;
 - d) identifying the gene as being up- or down-regulated during differentiation of the pPS cells if the frequency of transcripts in the library from the undifferentiated pPS cells is different from the frequency of transcripts in one or more libraries from the differentiated cell types at a statistical probability of at least 95%.
52. The method of claim 51, further comprising assessing a culture of pPS cells depending on the expression level measured in cells from the culture of the marker identified in d).
53. The method of any of claims 1-52, wherein the pPS cells are obtained from a human blastocyst, or are the progeny of such cells.
54. The method of claim 53, wherein the pPS cells are human embryonic stem cells.