

Immortalized neural stem cells differ from nonimmortalized cortical neurospheres and cerebellar granule cell progenitors

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Abstract

Pluripotent neural stem cells (NSCs) have been used as replacement cells in a variety of neurological disease models. Among the many different NSCs that have been used to date, most robust results have been obtained with the immortalized neural stem cell line (C17.2) isolated from postnatal cerebellum. However, it is unclear if other NSCs isolated from different brain regions are similar in their potency as replacement therapies. To assess the properties of NSC-like C17.2 cells, we compared the properties of these cells with those reported for other NSC populations identified by a variety of different investigators using biological assays, microarray analysis, RT-PCR, and immunocytochemistry. We show that C17.2 cells differ significantly from other NSCs and cerebellar granule cell precursors, from which they were derived. In particular, they secrete additional growth factors and cytokines, express markers that distinguish them from other progenitor populations, and do not maintain karyotypic stability. Our results provide a caution on extrapolating results from C17.2 to other nonimmortalized stem cell populations and provide an explanation for some of the dramatic effects that are seen with C17.2 transplants but not with other cells. We suggest that, while C17.2 cells can illustrate many fundamental aspects of neural biology and are useful in their own right, their unique properties cannot be generalized.

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Introduction

Neurogenesis in the developing embryo follows a characteristic pattern that is defined both spatially and temporally. Spatial domains are defined early in embryogenesis possibly as early as the process of neurulation. The prosencephalic, mesencephalic, and rhombencephalic separations take place early, and further subdivisions are

completed shortly thereafter. The initial wave of neurogenesis occurs from differentiating ventricular zone stem cells, while subsequent neuronal generation appears to be from subventricular zone cells. Neurons undergo tangential and radial migration along specified pathways and radial glia and are restricted to predefined domains. Neurogenesis continues in the postnatal period, and recent data have shown that this neurogenesis occurs via stem cells (Arlotta et al., 2003; Chmielnicki and Goldman, 2002; Gage, 2000; Limke and Rao, 2002; Temple, 2001). Many different stem cell populations have been described in the adult, including ventricular zone, subventricular zone, radial glial, astrocyte, parenchymal, and transdifferentiating cells (reviewed in Pevny and Rao, 2003). The

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properties of these cells differ from each other, although they all retain the property of multipotency and prolonged self-renewal.

The cerebellum, in contrast to most other CNS regions, develops postnatally. The cerebellar anlage develops from the rhombencephalon with the Purkinje cells developing early from the ventricular zone surrounding the fourth ventricle, while the granule cells are generated from a separate germ layer, the external rhombencephalic lip that extends over the forming cerebellum. Cells within this lip divide robustly to form the external granule cell layer. At around birth, these cells undergo maturation by the sequential expression of a series of transcriptional markers (reviewed in [Armstrong and Hawkes, 2000](#); [Millen et al., 1999](#); [Wassef and Joyner, 1997](#); [Wingate, 2001](#)). Cells migrate away from the external granule cell layer, although the residual process remains and constitutes the parallel fibers of the external granule cell layer that receive Purkinje cell synapses and ultimately come to reside below the Purkinje cells to form the internal granule cell layer. How similar these cerebellar progenitor/stem cells are or how they relate to cortical, ventricular zone, and subventricular zone stem cells remains unknown.

[Snyder et al. \(1992\)](#) have successfully immortalized dividing cells from the cerebellum at postnatal day 4 using a v-myc immortalizing oncogene ([Ryder et al., 1990](#)). The authors characterized several clones and showed that one, C17.2, fulfilled the criteria of a multipotent stem cell. Surprisingly, this line could readily differentiate into noncerebellar neurons, astrocytes, and oligodendrocytes both in vitro and in vivo ([Snyder et al., 1992, 1997](#); [Taylor and Snyder, 1997](#); [Vescovi and Snyder, 1999](#)). Equally importantly, its repertoire of differentiation appeared wider than that of its nonimmortalized cerebellar counterpart when transplanted into the hippocampus, striatum, or cortex, and C17.2 cells appeared to be able to respond in a site- and tissue-specific fashion to integrate seamlessly into the host environment ([Riess et al., 2002](#); [Yang et al., 2002](#)). Nevertheless, the cells retained the ability to respond to cerebellar cues and differentiate into granule cells as well ([Rosario et al., 1997](#); [Snyder et al., 1992](#)). Whether this expansion of its differentiation potential represented dedifferentiation, a by-product of immortalization, or simply revealed the intrinsic plasticity of all stem cells remains to be determined. It should be noted, though, that non-immortalized cerebellar granule cells would not differentiate into hippocampal neurons when transplanted in an identical fashion ([Alder et al., 1996](#); [Gao and Hatten, 1994](#)).

To assess the properties of C17.2 cells which have been used as a surrogate for (neural stem cells) NSCs isolated from different brain regions, we compared the properties of these cells with those reported for other NSC populations identified by a variety of different investigators using biological assays, microarray analysis, RT-PCR, and immunocytochemistry. We show that C17.2

cells differ significantly from other NSCs and retain some characteristics typical of granule cell precursors. In particular, they secrete many growth factors and cytokines that are not secreted by NSCs from other brain regions. Our results provide a caution on extrapolating results from C17.2 to other nonimmortalized stem cell populations and provide an explanation for some of the dramatic effects that are seen with C17.2 transplants but not with other cells. Furthermore, our results suggest that, while C17.2 NSCs may serve as a useful tool to deliver drugs and genes to a variety of targets, similar results cannot be expected from most other stem or progenitor cell populations utilized. Our results highlight the importance of careful side-by-side comparisons and the difficulties of extrapolating from superficially identical cells.

Materials and methods

Neurite outgrowth in organotypic spinal cord cultures

Organotypic spinal cord cultures were prepared from lumbar spinal cords of 8-day-old rat pups, as described previously ([Ho et al., 2000](#); [Rothstein et al., 1993](#)). Lumbar spinal cords were collected under sterile conditions and sectioned transversely into 350- μ m slices with a McIlwain tissue chopper. Slices were cultured on collagen (5 μ g/cm²)-coated Millicell CM semipermeable culture inserts at a density of five slices per well in an incubator at 37°C (5% CO₂, 95% humidity). Under these conditions, 95% of cultures retained cellular organization, and a stable population of motor neurons survived for more than 3 months. Culture media [50% minimal essential medium and HEPES (25 mM), 25% heat-inactivated horse serum, and 25% Hanks balanced salt solution (Gibco) supplemented with D-glucose (25.6 mg/ml), and glutamine (2 mM), at a final pH of 7.2] were changed twice weekly. After 7 days of culturing in the spinal cord media, the media were changed to conditioned media from C17.2 cells, control media for the C17.2 cells, conditioned media by E14.5 cortical neurospheres, or control media for the E14.5 cortical neurospheres. Cultures were fixed and stained with SMI-32 (anti-non-phosphorylated neurofilament antibody), as described before ([Ho et al., 2000](#)). Neurite outgrowth was quantified by counting the number of fibers exiting the spinal cord slices after 2 weeks in culture. The experiments were done three to six times, and the data from multiple slices were analyzed in Statview for Macintosh (v.5.0) using ANOVA with correction for multiple comparisons.

Neural stem cell culture

C17.2 cells were grown in an undifferentiated state in high-glucose DMEM supplemented with 10% fetal calf serum, 5% horse serum, and 2 mM glutamine on uncoated

tissue culture dishes in standard humidified 5% CO₂ at 37°C (Snyder et al., 1992). Cells were maintained in culture by feeding twice weekly with 1:1 mixture of conditioned medium from confluent C17.2 culture and fresh medium and were split 1:10 or 1:20 into fresh medium when confluent. Mouse E14.5 cortical neural stem cells (neurospheres) were purchased from StemCell Technologies (Vancouver, Canada) and cultured according to manufacturer's protocols (Cai et al., 2002). Ploidy analysis was done through the Johns Hopkins Karyotyping Facility. In brief, cells were fixed, and chromosome numbers and morphology were assessed by counting in at least 10 cells in each culture dish using classical cytogenetic banding techniques.

Microarray hybridization and data analysis

Total RNA was extracted from C17.2 NSCs, mouse cortical NSCs, and cerebellar granule cell neurons isolated from P4 C57BL/6J mice (same strain as C17.2 cells) using TRIZOL (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA was then quantified by absorption at 260 nm, and its integrity was assessed by electrophoresis through formamide/formaldehyde TAE gels.

Synthesis and labeling of cDNA and hybridization of cDNA probes to the GEArray™ cDNA expression arrays mouse Neurostem (MM-601.2) and Common Cytokines (MM-003) were performed according to the manufacturer's protocol (SuperArray Bioscience Corp., Frederick, MD). The biotin dUTP-labeled cDNA probes were specifically generated in the presence of a designed set of gene-specific primers according to protocols in Ampolabeling-LPR kit (SuperArray Inc.). The arrays were prehybridized at 60°C for 2 h and hybridized with biotin-labeled probes at 60°C for 16–20 h followed by four washes—first twice with 2 × SSC/1% SDS and then twice with 0.1 × SSC/1% SDS at 60°C for 15 min each. Chemiluminescent detection steps were performed at room temperature by subsequent incubation of the arrays with alkaline phosphatase-conjugated streptavidin and CDP-Star substrate (Applied Biosystems, Salt Lake City, UT) and exposure to X-ray film. The experiments were performed at least twice independently.

The imaging screens were scanned and analyzed with *ScanAlyze* 2.50 (Lawrence Berkeley National Lab, <http://www.microarrays.org/software.html>) and GEArray Analyzer (SuperArray Bioscience Corp.). For each sample, the pixel intensity of the genes spotted on the array filters was measured using *ScanAlyze* 2.50, and then the background was subtracted in GEArray Analyzer by subtracting average intensities derived from negative or blank spots. These resulting intensities were divided by the average of intensity from housekeeping genes, giving us the relative intensity for each spot. The positive and negative spots were independently identified and verified by at least two people. Only the

matched positive and negative results of two experiments are presented. Data from these membrane-based microarrays are very reliable and reproducible (Luo et al., 2003). Standard abbreviations for each gene were used in the tables. Full names of each gene are attached as an Appendix and are also available at <http://www.superarray.com>.

PCR primers and RT-PCR

The cDNA was synthesized using 2 µg of total RNA in the presence of Ready-to Go You Prime First Strand beads (Amersham) and random primers (Invitrogen). The mixtures were incubated at 37°C for 60 min, followed by a 10-min incubation at 90°C to inactivate the Superscript II. The cDNA was then diluted 10 times for future use. The PCR was performed in a total volume of 25 µl with 1 µl of 1:10 diluted cDNA, 1 × PCR buffer, 3 mM MgCl₂, 1 U Platinum Taq DNA polymerase (Invitrogen/Life Technologies), 0.2 mM dNTP (Promega), and 0.3 µM each of forward and reverse primers. The PCR was performed at 94°C for 5 min and then for 35 cycles at 94°C for 30 s, 55°C for 30 s, and 70°C for 30 s and a final extension for 10 min at 72°C. The primer sequences are available upon request.

Immunocytochemistry

Monoclonal antibodies anti-NCAM (clone 5A; dilution 1:5), anti-Nestin (dilution 1:5), and anti-Nkx2.2 (dilution 1:1) were purchased from Developmental Studies Hybridoma Bank. Monoclonal antibody clone A2B5 (clone 105) was purchased from ATCC, and supernatants were used at 1:10 dilution. Monoclonal antibody anti-CD44 (clone IM7) was kindly provided by Dr. Sherman and was used at 1:40 dilution. Anti-S100β (dilution 1:200) and anti-β-III tubulin (dilution 1:2000) antibodies were purchased from Sigma. Cultured cells were fixed with 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4) for 1 h at room temperature, permeabilized in 0.1% Triton X-100 for 10 min, blocked with 5% normal serum in 0.2% Triton X-100 for 1 h, and then incubated overnight at 4°C with the primary antibodies. The staining with A2B5 and anti-NCAM was done in live cells without fixation before the primary antibody. The staining was completed by incubation with either FITC- or cy3-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA), and the slides were mounted with Vectashield mounting medium with DAPI (Vector Laboratories). Appropriate controls included negative controls where primary antibodies were omitted and positive controls of tissue sections known to express the antigen under study. Sox2-EGFP mice were generated, as described previously (Ellis et al., in press). Postnatal day 4 Sox2-EGFP mice were perfused with 4% paraformaldehyde. Brains were removed and processed in successive sucrose gradients before freezing in OCT embedding solution. Cerebellar sections were cut at 8–12 µm and mounted, and photo-

graphs were taken using a digital camera attached to an Olympus microscope.

Results

Cortical NSCs and C17.2 cells differ in their ability to enhance motor axonal outgrowth and in the chemokines secreted

C17.2 cells have been used in a variety of injury paradigms and have given favorable therapeutic results (reviewed in Snyder et al., 2004). Several groups have extrapolated from these results to suggest that similar results could be obtained by NSCs isolated from other sources. To determine if C17.2 cells are similar to other NSCs, we first performed a side-by-side comparison of neuroregenerative potential of C17.2 cells, and neurospheres that were isolated from mouse E14.5 cortices (Fig. 1). We used a well-established model of motor axon regeneration from spinal cord explant cultures (Corse et al., 1999; Ho et al., 2000;

Rothstein et al., 1993). In this explant culture system, spinal cord explants from P8 rats survive for months, but unless given specific neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF), motor axons do not cross the white matter and exit the spinal cord explant (Ho et al., 2000).

To test if C17.2-conditioned medium provided trophic outgrowth, we collected medium conditioned by the C17.2 cells and compared its effects to conditioned medium from cortical neurospheres grown at the same cell density in similar culture conditions as well as control medium not conditioned by exposure to either cell population. Conditioned media from C17.2 cell cultures increased the number of motor axons traversing the inhibitory substrate of the white matter and exiting from the spinal cord explants (Fig. 1 and unpublished observations by Llado and Rothstein). In contrast, conditioned media from E14.5 cortical neurospheres or control nonconditioned medium had no effect on axonal outgrowth.

The difference in behavior of the two cell populations suggested that the pattern of cytokines secreted by these

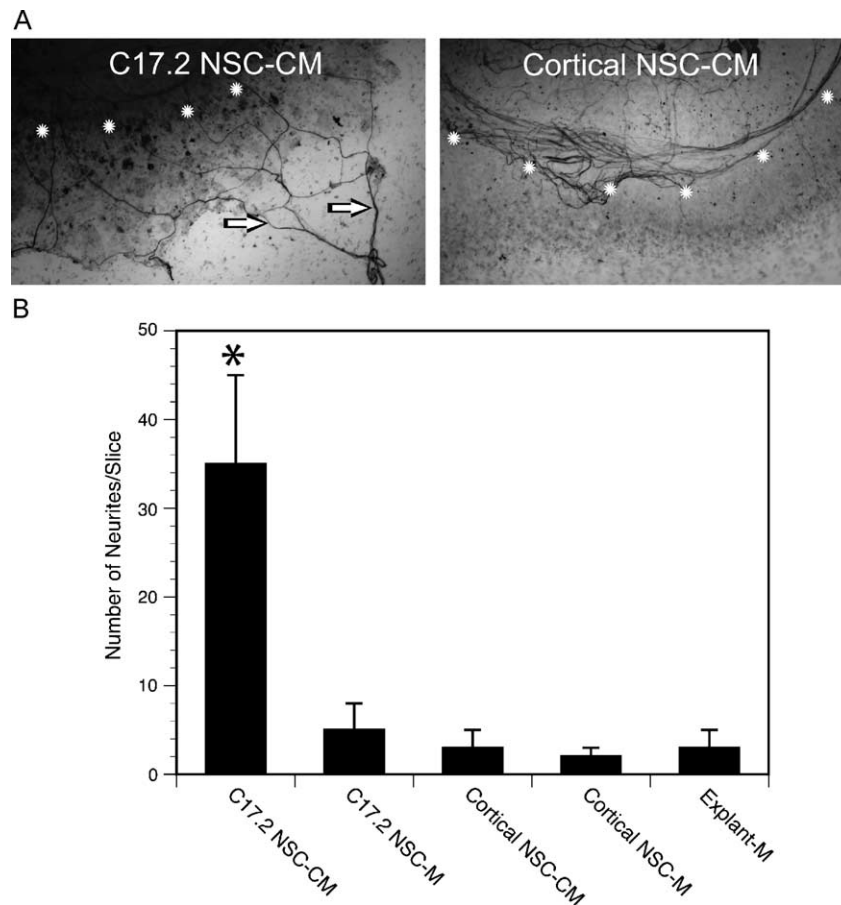


Fig. 1. Conditioned media from C17.2 cells induce axonal outgrowth from spinal cord explant cultures. Spinal cord explants from P8 rat pups were prepared as described, and after 1 week in culture, they were exposed to conditioned media from C17.2 cells or cortical NSCs or control media for 1 more week. (A) C17.2-conditioned media induced axonal outgrowth out of the gray matter (border delineated by stars) into the white matter and out of the explant (arrows in A). In contrast, cortical NSC-conditioned media had no effect on motor axons; they remained at the gray matter–white matter junction. (B) Quantitation of the number of axons exiting from the gray matter is shown. The number of axons per slice of spinal cord is expressed as an average of four to six slices per experiment done at least twice. (* $P < 0.001$ compared to the other conditions; NSC = neural stem cell, CM = conditioned media, M = nonconditioned media).

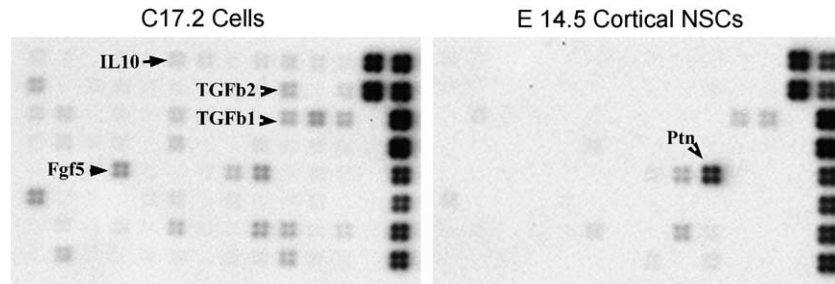


Fig. 2. Comparison of chemokine-related gene expression analysis between C17.2 cells and E14.5 cortical NSCs. Gene expression of undifferentiated C17.2 cells and neurospheres from E14.5 cortical NSCs were analyzed using focused chemokine microarray (Superarray, MM-003). Samples were harvested and processed in parallel to ensure uniformity. They were run in duplicate, and results presented are from experiments where identical results were obtained in duplicate runs. Representative images are shown. The data are summarized in Table 1.

cells might be different. To directly compare a subset of cytokines secreted by cells, we utilized a focused cytokine array and performed hybridization to assess the pattern of cytokine expression (Fig. 2 and Table 1). Samples were harvested and processed in parallel to ensure uniformity. Samples were run in duplicate, and results presented are from experiments where identical results were obtained in duplicate runs. As can be seen, the overall pattern of cytokine expression was quite different. C17.2 cells expressed a much larger repertoire of cytokines. In particular, members of the TGF- β family, GDFs, and BMP10 were expressed only by C17.2 cells, while, as previously noted, cortical NSCs express BDNF and other BMP family members. The difference in expression of GDFs may explain the differential response of motor axons to conditioned medium from C17.2 cells and neurospheres.

Patterns of gene expression differ between C17.2 cells and cortical NSCs

The dramatic difference in cytokine expression raised the possibility that other gene families may also be differentially expressed between C17.2 cells and other stem cell populations. In an attempt to compare overall expression

pattern, we examined the expression of stem cell-specific genes in C17.2 cells and E14.5 neurospheres using RT-PCR and a focused microarray described previously (Luo et al., 2003). RT-PCR of markers showed some similarities and many unexpected differences (Fig. 3). Similar to NSCs isolated from the neuroepithelium (Cai et al., 2003; Kalyani et al., 1998) and E14.5 neurospheres (see Fig. 3), undifferentiated C17.2 cells express Bcrp1, Cx43, Glut1, and TERT. Like cortical NSCs, C17.2 cells express EGFR and PDGFR α as well. Like cortical NSCs, C17.2 cells do not express markers of neuronal progenitors such as polysialated NCAM or glial progenitors such as A2B5 or NG2 (Fig. 3). However, unlike both neuroepithelial NSCs and neurosphere-derived NSCs, C17.2 cells express CD44, PLP/DM20, and S100 β indicative of a glial phenotype (Alfei et al., 1999; Mayer-Proschel et al., 1997; Raff et al., 1984). Unlike adult NSCs, however, C17.2 cells do not express GFAP or markers of neuroglial progenitors such as Olig1 and Olig2. Olig2 has been described as being expressed by dedifferentiated progenitor/stem cells in culture (Gabay et al., 2003; Liu and Rao, 2004).

Surprisingly, C17.2 cells do not express several markers characteristic of other NSCs. In particular, they do not express Brn1, Sox-1, or Sox-2. These markers are readily

Table 1
Gene expression profiles in mouse C17.2 cells and mouse E14.5 cortex neurosphere cells

Category	Detected only in mouse C17.2 cells but not in mouse E14.5 neurosphere cells (46)	Detected in both mouse C17.2 cells and mouse E14.5 neurosphere cells (77)	Detected only in mouse E14.5 neurosphere cells but not in mouse C17.2 cells (11)
Markers	Krt1-15; Myla; Ncam1; Pdgfrb; Sox10; Sox18 Tnc; Tubb3	Acta2; Actc1; Actg2; Cd44; Cnp1; Cst3; Egfr; Gcm2; Gjb1; Mtap1b; Myh11; Nkx2-2; Olig1; Pdx1; Pou3f2; Pou5f1; Prdc; Sox1; Sox2; Sox3; Sox4; Sox6; Tep1; Vim	Fabp7, Mbp; Olig2; Sox15
Cytokines, growth factors, and their receptors	Acvr2; Acvr11; Bmp10; Csf1; Epo; Fgf15; Fgf5; Fgfr1; Gdf1; Gdf11; Ifnb; Igf2r; Il10; Il16; Il17b; Il6st; Ins1; Ltb; Ngfr; Ptch; Tgfb1; Tgfb2; Tgfb3; Tnfsf4; Tnfsf6; Vegfb	Bmp1; BMP3; Bmp4; Bmp6; Bmp7; Bmpr1a; Bmpr2; Cntf; Fgf1; Fgf13; Fgf23; Fgf3; Fzd1; Fzd8; Gdf9; Ifnab; Ifrd1; Il2; Il7; Kitl; Notch2; Notch4; Ntf3; Shh; Tgfr1; Tgfr2; Tnfsf13b; Vegf; Vegfa; Wnt4; Wnt8a	BDNF; IL-18; Igfbp3; Ntrk2; PTN; Wnt3a
ECMs	Cdh2; Col6a2; Itga5; Itga6; Itgav; Itgb5	Catna1; Catna2; Catnal1; Catnb; Cdh15; Cdh3; Icam5; Itgae; Itgax; Itgb1	Cadherin 5
Others	Ceng2; Cdkn1a; Cdkn1b; Dnmt3a; Foxo1	Dnmt1; Erbb2ip; Erbb3; Foxm1; Inhbb; Nfkbia; Pten	

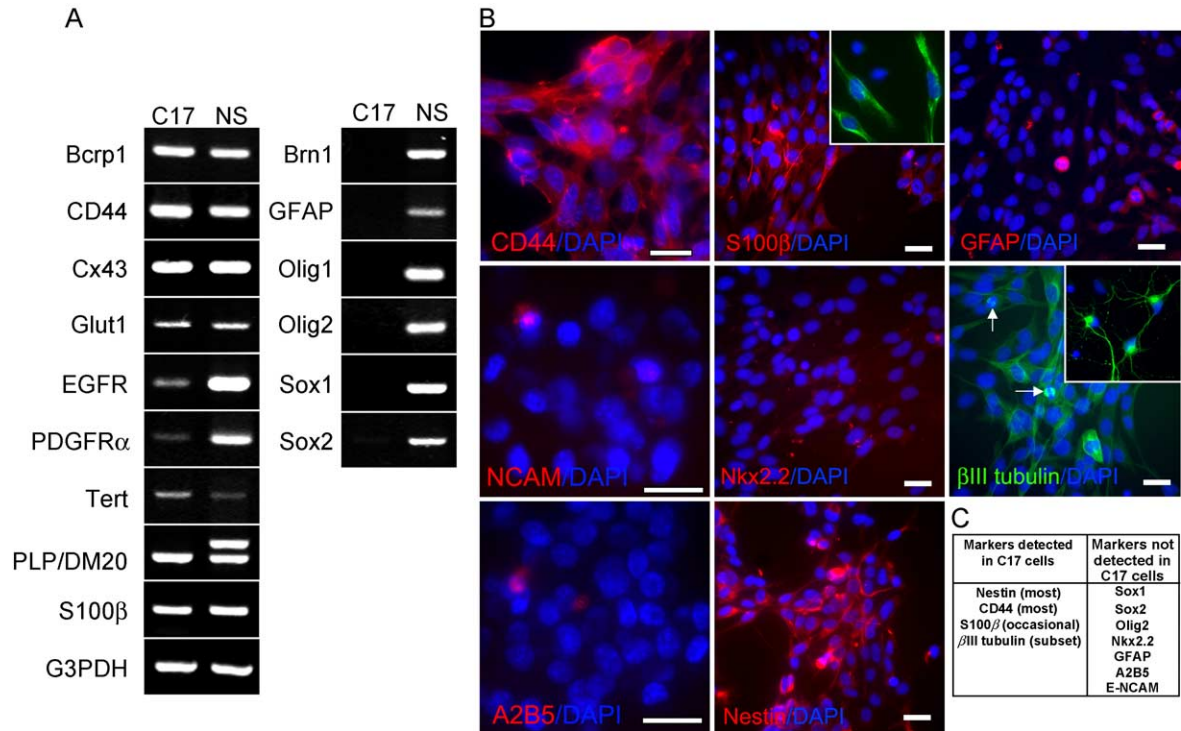


Fig. 3. RT-PCR and immunocytochemical analysis of a subset of genes examined by the microarrays. (A) Undifferentiated C17.2 cells express Bcrp1, CD44, Cx43, Glut1, EGFR, PDGFR α , Tert, PLP/DM20, and S100 β , but not Brn1, GFAP, Olig1, Olig2, Sox1, or Sox2 as detected by RT-PCR. Neurospheres (NS) from mouse cortical NSCs were used as positive control. (B) Most C17 cells express a surface marker CD44 and Nestin. A subset of C17.2 cells expresses neuronal marker β -III tubulin. However, the β -III tubulin pattern in C17.2 cells is quite different from typical neurons as shown in the inset. Arrows indicate that spindles are detected in occasional cells undergoing mitosis. Occasional S100 β -positive processes can be detected in C17.2 cells. Inset shows staining of S100 β expression in glial precursor cells. C17.2 cells do not express the neuronal precursor cell marker NCAM, glial precursor marker A2B5, oligodendrocyte precursor marker Nkx2.2, or astrocyte marker GFAP. Blue staining in each panel is DAPI. Scale bar = 25 μ m. (C) A summary list of markers detected or absent in C17 cells.

detected in cortical NSCs (Fig. 3). Unlike cortical NSCs, C17.2 cells appear to express a small amount of β -III tubulin, both by RT-PCR as well as by immunocytochemistry, although the pattern of β -III tubulin is different from the pattern seen in neurons. Overall, the RT-PCR data suggest that C17.2's are an undifferentiated population of cells that have a unique profile of neural stem cell markers which does not match the pattern described for either neuroepithelial stem cells, neurosphere type-NSCs, or GFAP-positive multipotential stem cells or of transdifferentiated progenitor cells. C17.2 cells express some glial markers consistent with previous reports that perinatal and adult stem cells may exhibit glial characteristics (Gotz and Steindler, 2003; Steindler and Laywell, 2003).

To further profile the similarities and/or differences between C17.2 cells and other NSC populations, we utilized a focused stem cell microarray (Fig. 4 and Table 1). This array contains 288 genes (240 unique genes) that represent a variety of growth factors, markers, and transcription factors thought to be expressed by stem and progenitor cells. We compared the pattern of expression seen with that of E14.5 neurosphere-derived NSCs. NSCs from E14.5 rodent cortical neural tissue appeared different in their overall pattern from C17.2 cells and resembled neuroepithelial stem cells to a large extent (Luo et al., 2002, 2003). The

microarray pattern of expression of C17.2 cells was confirmed by validating the expression of a subset of differentially expressed genes (data not shown). The number of genes that appeared differentially expressed (57) was larger than the number that differed between cerebellar granule cells and C17.2 cells (see below) and represented a large fraction of the total number of expressed genes (about

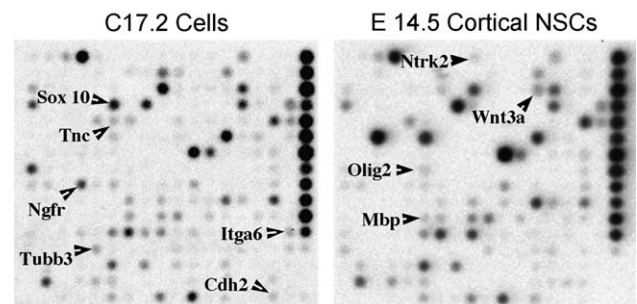


Fig. 4. Comparison of stem cell-related gene expression analysis between C17.2 cells and E14.5 cortical NSCs. Gene expression of undifferentiated C17.2 cells and neurospheres from E14.5 cortical NSCs were analyzed using focused stem cell microarray (Superarray, MM-601.2). Samples were harvested and processed in parallel to ensure uniformity. They were run in duplicate, and results presented are from experiments where identical results were obtained in duplicate runs. Representative images are shown. The data are summarized in Table 1.

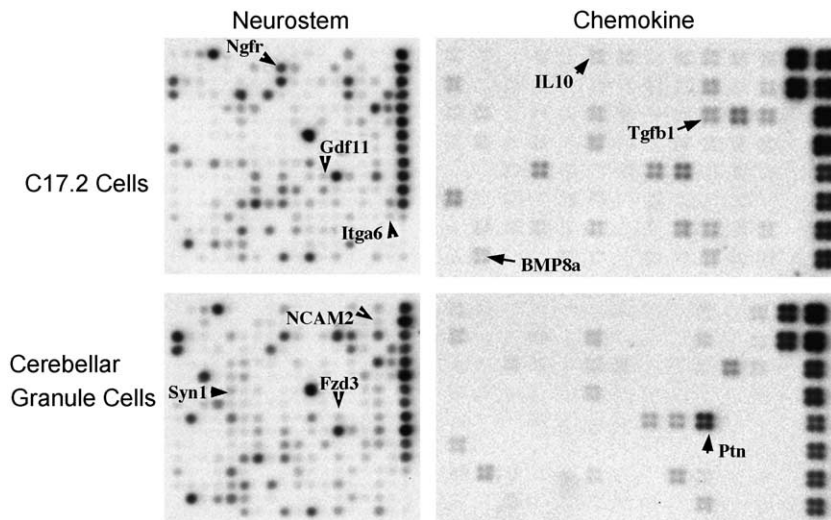


Fig. 5. Comparison of stem cell-related gene expression analysis between C17.2 cells and cerebellar granule cells. Gene expression of undifferentiated C17.2 cells and cerebellar granule cells were analyzed using focused chemokine microarray (Superarray, MM-003) and stem cell microarray (Superarray, MM-601.2). Samples were harvested and processed in parallel to ensure uniformity. They were run in duplicate, and results presented are from experiments where identical results were obtained in duplicate runs. Representative images are shown. The data are summarized in Table 2.

20%) and confirmed and extended the differences observed by RT–PCR. Thus, both RT–PCR of a subset of genes and a more global comparison indicated that C17.2 cells are an undifferentiated population of cells that do not strongly resemble any specific neural stem cell population.

C17.2 cells do not resemble cerebellar granule cells

Since C17.2 cells were generated from postnatal mouse cerebellar granule neurons, it is possible that the expression profile of C17.2 cells is more closely related to that of cerebellar granule neurons isolated from mice at the same age. Cerebellar granule cell precursors are dividing at the stage at which immortalization was performed and C17.2

cells can generate cerebellar granule cells upon transplantation (Alder et al., 1996). C17.2 cells express β -III tubulin at low levels as well.

We therefore used the same focused microarrays to directly compare the pattern of gene expression between cerebellar granule cells and C17.2 cells (Fig. 5 and Table 2). The number of genes that were unique to C17.2 cells was lower compared to the differences between C17.2 cells and cortical NSCs (Table 2). Nevertheless, there were many differences among the genes expressed by C17.2 cells and the cerebellar granule cell precursors used in this experiment.

Given the closer similarity of C17.2 cells to cerebellar granule cells than to cortical NSCs, we wondered if the properties of C17.2 reflect the properties of cerebellar granule

Table 2
Gene expression profiles in mouse C17.2 cells and mouse cerebellar granular cells

Category	Detected only in mouse C17.2 cells but not in mouse cerebellar granule cells (21)	Detected in both mouse C17.2 cells and mouse cerebellar granule cells (106)	Detected only in mouse cerebellar granule cells but not in mouse C17.2 cells (28)
Markers	Myla; Pdgfrb; Sox18	Acta2; Actc1; Actg2; Cd44; Cnp1; Cst3; Egfr; Gcm2; Gjb1; Krt1-15; Mtap1b; Myh11; Ncam1; Nes; Nkx2-2; Olig1; Pdx1; Pou3f2; Pou3f3; Pou5f1; Prdc; S100B; Sox1; Sox10; Sox2; Sox3; Sox4; Sox6; Tep1; Tnc; Tubb3; Vim	Fabp7; Ina; Mtab2; Ncam2; Pax6; Pdgfra; Slc1a2; Sox15; Sox5; Syn1
Cytokines, growth factors, and their receptors	Acvr2; Bmp10; Bmp8a; Epo; Fgf1; Fgf3; Gdf1; Gdf11; Il10; Il17b; Ltb; Ngfr; Tgfb1; Tgfb1r; Tnfsf4; Tnfsf6; Wnt11	Acvr1; Bmp1; BMP3; Bmp4; Bmp6; Bmp7; Bmpr1a; Bmpr2; Cntf; Csf1; Fgf13; Fgf15; Fgf17; Fgf23; Fgf3; Fgf5; Fgfr1; Fzd1; Gdf9; Ifnab; Ifrd1; Igf1; Igf2r; Il2; Il6st; Il7; Ins1; Kitl; Ngfr; Nodal; Notch2; Notch4; Ntf3; Pdgfa; Ptch; Shh; Tgfb2; Tgfb2r; Tnfsf13b; Vegf; Vegfa; Vegfb; Vegfc; Wnt4; Wnt6; Wnt8a	Aif1; Cntfr; Fgf2; Fgf9; Fzd3; Ifna1; Igf2; Il16; Notch1; Ntrk2; Ptn; Wnt3a
ECMs	Itga6	Catna1; Catna2; Catnal1; Catnb; Cdh15; Cdh2; Cdh3; Col6a2; Icam5; Itga5; Itgae; Itgav; Itgax; Itgb1; Itgb5	Catnd2; Cdh4; Cdh5; Vcam1; Fabp7
Others		Ceng2; Cdkn1a; Cdkn1b; Dnmt1; Dnmt3a; Dnmt3l; Erbb2ip; Erbb3; Foxm1; Foxo1; Nfkbia; Pten	Erbb4

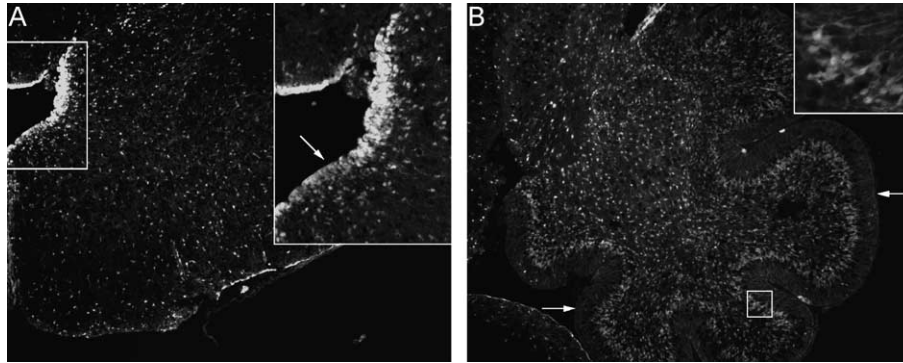


Fig. 6. Expression of Sox-2 in mouse P4 cerebellum. Sections from immature (A) and more mature region (B) of the P4 mouse cerebellum are shown. GFP expression is seen in the external granule cell layer and is downregulated as the cells migrate to their final positions, although subsets of cells are still positive even in the inner granule cell layer (inset in B). No expression is seen in Bergman glia or in Purkinje cells.

cell progenitors. An important potential distinction between C17.2 cells and other stem cell populations was the absence of Sox-1 and Sox-2 expressions in the C17.2 cells. Therefore, we asked if cerebellar granule progenitors also failed to express these genes. RT-PCR (data not shown) indicated, however, that both Sox-1 and Sox-2 were expressed by cerebellar granule cell precursors. To confirm that this was true *in vivo*, we took advantage of the Sox-2 GFP transgenic mouse, which expresses GFP under the Sox-2 promoter (Ellis et al., *in press*). As in other brain regions, Sox-2 GFP expression was present in the ventricular zone and subventricular zone regions (data not shown; however, see Ellis et al., *in press*). In the cerebellum, GFP expression was also seen in the external granule cell layer but was later downregulated as the cells migrated to their final positions (Fig. 6 and Ellis et al., *in press*). No expression of Sox-2/GFP was seen in Bergman glia or in Purkinje cells. Thus, the failure of C17.2 expression cannot be attributed to the absence of Sox gene expression in this region of the brain. It is more likely that either Sox-1 or Sox2 expression was lost as cells were maintained in culture or that the cellular origin of this immortalized multipotential cell is the Bergman glial cell or a dedifferentiated progenitor cell.

C17.2 cells are aneuploid

The multiple differences observed between C17.2 cells and other stem cell populations including cerebellar granule cell precursors raised the possibility that some of the differences may be attributed to changes in the cells after immortalization and as they adapted to culture. A common change in immortalized cell population is a loss of euploidy and the divergence of cell properties from the parent cell population. This has been seen in multiple lines, and variants with dramatically different properties can often be isolated. Indeed, PC12 variants are one such example. To determine if this may explain the divergence in properties of C17.2 cells, we undertook a karyotype analysis of the different clones available to us (passage numbers 40–60). Fig. 7 shows two representative samples of a standard ploidy analysis of 10

metaphase spreads of C17.2 NSCs grown under undifferentiated conditions. All of the cells undergoing division were aneuploid with most having chromosome numbers around 60. In half of the metaphase spreads, there were chromosomes fused at the centromeres, suggesting loss of telomeric ends and other changes characteristic of cells propagated in culture for prolonged periods. Thus, at least some of the differences in properties between C17.2 cells and other nonimmortalized populations may arise from changes during immortalization and loss of euploidy.

Discussion

Pluripotent NSCs have been used as replacement cells in a variety of neurological disease models. Among the many different NSCs that have been used to date, most robust results have been obtained with the immortalized C17.2 cell line isolated from postnatal cerebellum. However, the genes that confer these therapeutic properties are unknown. Here, we show that these cells express both neural stem cell markers as well as markers of more differentiated cells along the glial pathway. We also show that their expression profile is different from that of nonimmortalized neurospheres isolated from E14.5 mouse cortices and also from that of the

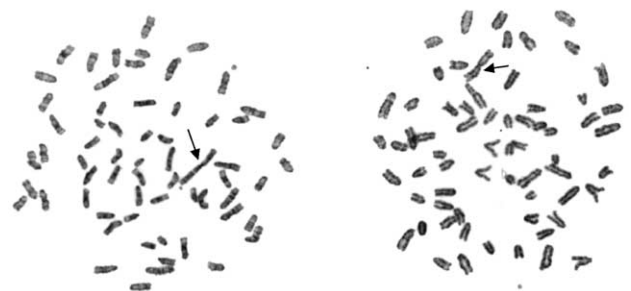


Fig. 7. Ploidy analysis of undifferentiated C17.2 cells. Two representative metaphase spreads of undifferentiated C17.2 cells are shown. All of the examined metaphase spreads showed aneuploidy with chromosome numbers around 60. Arrows point to chromosomes fused at the centromeres.

nonimmortalized cerebellar granule neurons that they were isolated from.

Cell lines have proven to be of immense utility in dissecting out biological pathways and understanding the behavior of difficult to obtain primary cells. PC12, Hek293, NTera2's and others are examples of widely used cell lines that have proven their utility. We believe that C17.2 cells are a useful surrogate for difficult to obtain multipotent neural stem cell populations. Like cortical NSCs, C17.2 cells express few markers of differentiation, can be readily propagated and genetically modified, and are freely available to all investigators through the generosity of the investigators responsible for generating this clone. C17.2 cells have illustrated several fundamental biological processes such as directed migration (Snyder et al., 1992, 1995) and homing (Liu et al., 1999) and have provided direct demonstration of the ability of the brain to direct site- and region-specific differentiation and repair (Lu et al., 2003; Riess et al., 2002; Yang et al., 2002).

Our present results do not detract from the utility of C17.2 cells, but rather interject a note of caution into directly extrapolating from results obtained with this cell line to primary rodent stem cells and to human stem cell populations which differ in significant ways from their nonimmortalized rodent counterparts (see Results). Our basis for recommending such caution comes from direct comparisons between C17.2 cells and neurosphere-forming cortical NSCs that are the most commonly used neural stem cell population. Our results show significant differences between these populations in growth factors released, ability to direct migration, their karyotype, and pattern of markers expressed.

One mechanism by which NSCs or other progenitor cells may provide therapeutic utility is to deliver trophic factors in a localized site. Indeed, this potential has been widely discussed and is in part based on the dramatic ability of C17.2 cells to direct motor neuron outgrowth in explant cultures and in spinal cord injury models (Lu et al., 2003). Our results suggest that a similar result will not be obtained with cortical NSCs as, in a side-by-side comparison, the two cells types behaved differently. A possible basis for the difference may be the secretion of GDNF, which is known to promote directed outgrowth from motor neurons (Oppenheim et al., 1995; Yan et al., 1995; Zurn et al., 1994).

The difference in the pattern of cytokines expressed raised the possibility that C17.2 cells and cortical NSCs may not be as similar as previously supposed despite the many markers they have been reported to share and the similarity in their multipotential ability. To test this hypothesis, we compared expression of stem cells markers in neurosphere-type stem cells, neuroepithelial-type stem cells, and C17.2 cells. Several differences were observed (see Results) providing support for our impression that these populations may have distinct biological properties. Of importance was the demonstration that C17.2 cells do not express detectable levels of Sox-1 and Sox-2 as assessed by RT-PCR. Expression of Sox-1 and Sox-2 has been almost universal in stem/precursor cell populations from the ventricular and

subventricular zones. Absence of both of these markers in C17.2 cells suggests that C17.2 cell line is not similar to the other neural stem cell populations commonly isolated from ventricular or subventricular zones in different brain regions as well. Our analysis of Sox expression in the cerebellum shows that Sox2 and Sox1 (data not shown) show that Sox genes are expressed in the cerebellar ventricular zone as well as in the external granule cell layer but are rapidly downregulated as cells migrate to the internal granule cell layer. No expression is seen in Bergman glia and in Purkinje cells. This raises the possibility that C17.2 cells were derived from Bergman glia or transdifferentiated or dedifferentiated cells or that C17.2 cells have diverged in culture and altered gene expression significantly. We tend to favor latter possibility, as the predominant population of dividing cells at the stage when C17.2 cells were derived is the granule cell precursor. Bergman glial cells do not culture well and in general do not divide extensively, a prerequisite for successful immortalization. Furthermore, granule cell precursors are limited to generating neurons *in vivo*; immortalization has been shown to expand their repertoire for differentiation (Gao and Hatten, 1994). Indeed, Hatten et al., in direct side-by-side experiments, showed that non-immortalized granule cell precursors from the cerebellum failed to differentiate into hippocampal neurons, while their immortalized counterparts readily did. This possibility is consistent with early reports that C17.2 cells seemed far more capable of differentiating into neurons than neurosphere cultures (our unpublished results) and that glial differentiation has been difficult to obtain.

Our results show that, as is common with many immortalized cell populations, C17.2 cells are aneuploid. Karyotyping of the clone maintained in our laboratory showed that most cells were aneuploid and had an average chromosomal number of 60. Overall, cells showed a similar pattern of chromosomal abnormality suggesting that this is a stable phenotype. At this stage, we cannot determine when this abnormality arose and whether all available clones of C17.2 bear this abnormality. However, irrespective of whether multiple variants of C17.2 exist in different laboratories (as with PC12 cells) or if this abnormality arose early and is common to all vials of C17.2 cells, it is clear that caution must be exercised in comparing results of transplantation, patterns of gene expression, or cellular behavior between immortalized and nonimmortalized cell populations. In particular, the dramatic migration ability of C17.2 cells may not necessarily reflect the potential of nonimmortalized NSC cells. We would encourage other users of this cell line to test karyotype to determine if multiple variants of this line exist and whether one can compare results across laboratories where C17.2 cells were used in a particular experiment. We note that widely differing results have been reported on differentiation in different laboratories. For example, when transplanted to intact or lesioned striatum, C17.2 cells spontaneously differentiated into dopaminergic neurons in one laboratory

(Yang et al., 2002). Yet in another laboratory, when transplanted into normal or lesioned spinal cord, majority of C17.2 cells either remained undifferentiated or differentiated into GFAP-positive astrocytes (Llado et al. unpublished results). These differing results from different laboratories are perhaps indicative of variations in the subclones of C17.2 cells. It is important to emphasize that karyotypic stability is an issue not just with immortalized populations but with any cell maintained in culture for prolonged time periods and has been emphasized for ES cells in particular (Carpenter et al., 2004; Rosler et al., 2004).

In summary, our results highlight the importance of a careful assessment of the cellular phenotype used in any experiment including a detailed analysis of marker gene expression, differentiation ability, and karyotype of any cell maintained in long-term culture. Our results comparing C17.2 cells with cortical NSCs suggest that this immortalized clone is a tremendously useful tool for assessment of specific

aspects of multipotential cell biology. However, not all results utilizing this population can be extrapolated to using NSCs of any kind. Our results provide a basis for the number of differences reported and raise caution in comparing results between laboratories and across cell types without a detailed side-by-side comparison.

Acknowledgments

We gratefully acknowledge the input of all members of our laboratories provided through discussions and constructive criticisms. Tobi L. Limke was supported by a Pharmacology Research Associate (PRAT) fellowship from NIGMS (NIH). Mahendra S. Rao was supported by the NIA (NIH), the Packard Center for ALS Research at Johns Hopkins and the CNS foundation. Ahmet Höke was supported by the NINDS, NIMH (NIH), and the Packard Center for ALS Research at Johns Hopkins.

Appendix A. GEArray S series mouse stem cell gene array (Mm-601.2)

Array Layout

Abcg2	Acta2	Actc1	Actg2	Acvr1	Acvr2	Acvr1l	Alb1	Anf-ESTs	Bdnf	Bmp1	Bmp10	Bmp15	Bmp2	Bmp3	Bmp4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bmp5	Bmp6	Bmp7	Bmp8a	Bmp8b	Bmpr1a	Bmpr1b	Bmpr2	Catna1	Catna2	Catnal1	Catnb	Catnd2	Ccng2	Cd34	Cd44
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Cdh1	Cdh15	Cdh2	Cdh3	Cdh4	Cdh5	Cdkn1a	Cdkn1b	Cdkn2d	Cer1	Cnp1	Cntf	Cntfr	Col6a2	Cst3	Dlk1
33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Dnmt1	Dnmt2	Dnmt3a	Dnmt3b	Dnmt3l	Drng11	Egfr	Egfr	Egr2	Erbp2ip	Erbp3	Erbp4	Fabp4	Fabp7	Fgf1	Fgf10
49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
Fgf11	Fgf12	Fgf14	Fgf15	Fgf16	Fgf17	Fgf18	Fgf2	Fgf20	Fgf21	Fgf22	Fgf23	Fgf3	Fgf4	Fgf5	Fgf6
65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Fgf7	Fgf8	Fgf9	Fgfr1	Fgfr2	Fgfr3	Fgfr4	Foxa1	Foxg1	Foxh1	Foxm1	Foxo1	Fzd1	Fzd3	Fzd4	Fzd7
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
Fzd8	Fzd9	Gata2	Gata4	Gcg	Gcm2	Gdf1	Gdf11	Gdf2	Gdf3	Gdf5	Blank	Gdf8	Gdf9	Gfap	Gja7
97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112
Gjb1	Gjb3	Gjb4	Gjb5	Icam1	Icam5	Igf1	Igf1r	Igf2	Igf2r	Igf3	Il6	Il6ra	Il6st	Ina	Inhba
113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128
Inhbb	Ins1	Insrr	Isl1	Itga2	Itga2b	Itga3	Itga4	Itga5	Itga6	Itga7	Itga8	Itgae	Itgal	Itgam	Itgav
129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
Itgax	Itgb1	Itgb2	Itgb3	Itgb4	Itgb5	Itgb6	Itgb7	F11r	Kdr	Krt1-14	Krt1-15	Krt1-17	Krt1-5	Krt2-8	Lif
145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
Lifr	Mbp	Mtap2	Mtap1b	Myh11	Myh6	Myl4	Ncam1	Ncam2	Nes	Neurog1	Nefl	Ngfb	Ngfr	Nkx2-	Nkx2-5
161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
Nodal	Nog	Notch1	Notch2	Notch3	Notch4	Odz4	Nrg3	Nrg4	Ntf3	Ntrk2	Ntrk3	Numb	Olig1	Olig2	Pax6
177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192
Pdgfa	Pdgfb	Pdgfra	Pdgfrb	Ipfl	Pecam	Plp	Pou3f2	Pou3f3	Pou5f1	Pou6f1	Prdc	Prom	Prox1	Ptch	Ptch2
193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208
Pten	Ptprc	S100b	Stmn2	Shh	Slc1a2	Slc1a6	Slc2a1	Snai1	Snai2	Sox1	Sox10	Sox13	Sox15	Sox17	Sox18
209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224
Sox2	Sox3	Sox4	Sox5	Sox6	Sox9	Syn1	Tebp-pend-	Tep1	Terf1	Tert	Tgfb1	Tgfb2	Tgfb3	Tgfb1	Tgfb2
225	226	227	228	229	230	231	vim 232	233	234	235	236	237	238	239	240
Tgfb3	Thy1	Tnc	Tubb3	Utf1	Vcam1	Vegfa	Vim	Wnt11	Wnt2	Wnt3a	Wnt4	Wnt5b	Wnt6	Wnt7b	Wnt8a
241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256
Zfp110	Zfp42	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	PUC18	PUC18	PUC18	PUC18
257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272
Rpl13a	Rpl13a	Rpl13a	Rpl13a	Gapd	Gapd	Gapd	Gapd	Ppia	Ppia	Ppia	Ppia	Actb	Actb	Actb	Actb
273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288

Gene Table

Position	Unigene	GeneBank	Symbol	Description	Gene name
1	Mm.333096	NM_011920	Abcg2	ATP-binding cassette, subfamily G (WHITE), member 2	Abcg2
2	Mm.213025	NM_007392	Acta2	Actin, alpha 2, smooth muscle, aorta	“actin, alpha 2,”
3	Mm.686	NM_009608	Actc1	Actin, alpha, cardiac	Actc1
4	Mm.292865	NM_009610	Actg2	Actin, gamma 2, smooth muscle, enteric	Actg2
5	Mm.689	NM_007394	Acvr1	Activin A receptor, type 1	TSK-7L
6	Mm.314338	NM_007396	Acvr2	Activin receptor IIA	AcvR2
7	Mm.279542	NM_009612	Acvr11	Activin A receptor, type II-like 1	ALK1
8	Mm.16773	NM_007423	Alb1	Albumin 1	Alb1,Afp
9	Mm.19961	AA036281	Anf-ESTs	Similar to atrial natriuretic peptide precursor-mouse	ANF
10	Mm.1442	NM_007540	Bdnf	Brain derived neurotrophic factor	BdNF
11	Mm.27757	NM_009755	Bmp1	Bone morphogenetic protein 1	BmP1
12	Mm.349334	NM_009756	Bmp10	Bone morphogenetic protein 10	BmP10
13	Mm.42160	NM_009757	Bmp15	Bone morphogenetic protein 15	BmP15/GDF9B
14	Mm.235230	NM_007553	Bmp2	Bone morphogenetic protein 2	BmP2
15	Mm.209571	NM_173404	Bmp3	Bone morphogenetic protein 3	BmP3
16	Mm.6813	NM_007554	Bmp4	Bone morphogenetic protein 4	BmP 4
17	Mm.118034	NM_007555	Bmp5	Bone morphogenetic protein 5	BmP 5
18	Mm.254978	NM_007556	Bmp6	Bone morphogenetic protein 6	BmP6
19	Mm.595	NM_007557	Bmp7	Bone morphogenetic protein 7	BmP 7
20	Mm.270287	NM_007558	Bmp8a	Bone morphogenetic protein 8a	BmP 8a, Oxct2a
21	Mm.30413	NM_007559	Bmp8b	Bone morphogenetic protein 8b	BmP8b/OP-3
22	Mm.237825	NM_009758	Bmpr1a	Bone morphogenetic protein receptor, type 1A	ALK-3
23	Mm.39089	NM_007560	Bmpr1b	Bone morphogenetic protein receptor, type 1B	Alk-6
24	Mm.7106	NM_007561	Bmpr2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	BMPR2
25	Mm.18962	NM_009818	Catna1	Catenin alpha 1	Catna1
26	Mm.34637	NM_009819	Catna2	Catenin alpha 2	Catna2
27	Mm.218891	NM_018761	Catna11	Catenin alpha-like 1	Catna11
28	Mm.291928	NM_007614	Catnb	Catenin beta b	b Catenin
29	Mm.6680	NM_008729	Catnd2	Catenin delta 2	Ctnd2
30	Mm.3527	NM_007635	Ccng2	Cyclin G2	Cyclin G2
31	Mm.29798	NM_133654	Cd34	CD34 antigen	Cd34
32	Mm.330428	M27130	Cd44	CD44 antigen	CD44
33	Mm.35605	NM_009864	Cdh1	Cadherin 1	E-cadherin
34	Mm.1976	NM_007662	Cdh15	Cadherin 15	M-cadherin
35	Mm.257437	NM_007664	Cdh2	Cadherin 2	Cadherin 2
36	Mm.4658	XM_134405	Cdh3	Cadherin 3	Cadherin 3
37	Mm.184711	NM_009867	Cdh4	Cadherin 4	Cadherin 4
38	Mm.21767	NM_009868	Cdh5	Cadherin 5	Cadherin 5
39	Mm.195663	NM_007669	Cdkn1a	Cyclin-dependent kinase inhibitor 1A (P21)	p21Waf1/p21cip
40	Mm.2958	NM_009875	Cdkn1b	Cyclin-dependent kinase inhibitor 1B (P27)	p27Kip1
41	Mm.29020	NM_009878	Cdkn2d	Cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	p19
42	Mm.6780	NM_009887	Cer1	Cerberus 1 homolog (<i>Xenopus laevis</i>)	CER1
43	Mm.15711	NM_009923	Cnp1	Cyclic nucleotide phosphodiesterase 1	Cnp1
44	Mm.290924	NM_053007	Cntf	Ciliary neurotrophic factor	CNTF/Zfp91
45	Mm.272210	NM_016673	Cntfr	Ciliary neurotrophic factor receptor	CNTFR
46	Mm.1949	NM_146007	Col6a2	Procollagen, type VI, alpha 2	Col6a2
47	Mm.4263	NM_009976	Cst3	Cystatin C	Cystatin C
48	Mm.157069	NM_010052	Dlk1	Delta-like 1 homolog (<i>Drosophila</i>)	DLK
49	Mm.128580	NM_010066	Dnmt1	DNA methyltransferase (cytosine-5) 1	DNMT1
50	Mm.6979	NM_010067	Dnmt2	DNA methyltransferase 2	Dnmt2
51	Mm.5001	NM_007872	Dnmt3a	DNA methyltransferase 3A	Dnmt3a
52	Mm.330894	NM_010068	Dnmt3b	DNA methyltransferase 3B	DNMT3b
53	Mm.13433	NM_019448	Dnmt3l	DNA (cytosine-5-)-methyltransferase 3-like	Dnmt3l
54	Rn.10189	NM_145767	Drg11	Paired-like homeodomain transcription factor	DRG11
55	Mm.254772	NM_010113	Egf	Epidermal growth factor	EGF
56	Mm.8534	NM_007912	Egfr	Epidermal growth factor receptor	EGFR
57	Mm.290421	NM_010118	Egr2	Early growth response 2	Krox-20
58	Mm.277354	NM_021563	ErbB2ip	ErbB2 interacting protein	ErbB2ip
59	Mm.29023	XM_125954	ErbB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	ErbB3
60	Mm.344033	XM_136682	ErbB4	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	ErbB4
61	Mm.582	NM_024406	Fabp4	Fatty acid binding protein 4, adipocyte	Fabp4
62	Mm.3644	NM_021272	Fabp7	Fatty acid binding protein 7, brain	Fabp7

(continued on next page)

Gene Table (continued)

Position	Unigene	GeneBank	Symbol	Description	Gene name
63	Mm.241282	NM_010197	Fgf1	Fibroblast growth factor 1	aFGF
64	Mm.317323	NM_008002	Fgf10	Fibroblast growth factor 10	FGF10
65	Mm.269011	NM_010198	Fgf11	Fibroblast growth factor 11	FGF11
66	Mm.7996	NM_010199	Fgf12	Fibroblast growth factor 12	FGF12A
67	Mm.32472	NM_010201	Fgf14	Fibroblast growth factor 14	FGF14 (FHF4)
68	Mm.3904	NM_008003	Fgf15	Fibroblast growth factor 15	FGF15 = Huma Fgf19
69	Mm.154768	NM_030614	Fgf16	Fibroblast growth factor 16	FGF16
70	Mm.12814	NM_008004	Fgf17	Fibroblast growth factor 17	FGF17
71	Mm.246671	NM_008005	Fgf18	Fibroblast growth factor 18	FGF18
72	Mm.57094	NM_008006	Fgf2	Fibroblast growth factor 2	bFGF
73	Mm.348043	NM_030610	Fgf20	Fibroblast growth factor 20	FGF20
74	Mm.143736	NM_020013	Fgf21	Fibroblast growth factor 21	FGF21
75	Mm.154211	NM_023304	Fgf22	Fibroblast growth factor 22	FGF22
76	Mm.347933	NM_022657	Fgf23	Fibroblast growth factor 23	FGF23
77	Mm.4947	NM_008007	Fgf3	Fibroblast growth factor 3	FGF3(int-2)
78	Mm.4956	NM_010202	Fgf4	Fibroblast growth factor 4	FGF4
79	Mm.5055	NM_010203	Fgf5	Fibroblast growth factor 5	FGF5
80	Mm.3403	XM_132863	Fgf6	Fibroblast growth factor 6	FGF6
81	Mm.330557	NM_008008	Fgf7	Fibroblast growth factor 7	FGF7/KGF
82	Mm.4012	NM_010205	Fgf8	Fibroblast growth factor 8	FGF8
83	Mm.8846	NM_013518	Fgf9	Fibroblast growth factor 9	FGF9
84	Mm.265716	NM_010206	Fgfr1	Fibroblast growth factor receptor 1	FLG
85	Mm.16340	NM_010207	Fgfr2	Fibroblast growth factor receptor 2	FGFR2 (KGFER)
86	Mm.6904	NM_008010	Fgfr3	Fibroblast growth factor receptor 3	FGFR3
87	Mm.276715	NM_008011	Fgfr4	Fibroblast growth factor receptor 4	FGFR4
88	Mm.4578	NM_008259	Foxa1	Forkhead box A1	Foxa1
89	Mm.4704	NM_008241	Foxg1	Forkhead box G1	Hfbbf1
90	Mm.42011	NM_007989	Foxh1	Forkhead box H1	Fast2
91	Mm.42148	NM_008021	Foxm1	Forkhead box M1	MPP2
92	Mm.29891	NM_019739	Foxo1	Forkhead box O1	FKHR1
93	Mm.246003	NM_021457	Fzd1	Frizzled homolog 1 (<i>Drosophila</i>)	Fzd1
94	Mm.243722	NM_021458	Fzd3	Frizzled homolog 3 (<i>Drosophila</i>)	Fzd3
95	Mm.86755	NM_008055	Fzd4	Frizzled homolog 4 (<i>Drosophila</i>)	Fzd4
96	Mm.297906	NM_008057	Fzd7	Frizzled homolog 7 (<i>Drosophila</i>)	Fzd7
97	Mm.184289	NM_008058	Fzd8	Frizzled homolog 8 (<i>Drosophila</i>)	Fzd8
98	Mm.6256	XM_284144	Fzd9	Frizzled homolog 9 (<i>Drosophila</i>)	Fzd9
99	Mm.272747	NM_008090	Gata2	Gata2 GATA binding protein 2	Gata2
100	Mm.161558	NM_008092	Gata4	GATA-binding transcription factor	GATA4
101	Mm.45494	NM_008100	Gcg	Glucagon	Gcg
102	Mm.1399	NM_008104	Gcm2	Glial cells missing homolog 2 (<i>Drosophila</i>)	Gcm2
103	Mm.348055	NM_008107	Gdf1	Growth differentiation factor 1	GDF1
104	Mm.299218	AF092734	Gdf11	Growth differentiation factor 11	BmP11/GDF11
105	Mm.116788	NM_019506	Gdf2	Growth differentiation factor 2	BmP9/GDF2
106	Mm.299742	NM_008108	Gdf3	Growth differentiation factor 3	GDF3
107	Mm.4744	NM_008109	Gdf5	Growth differentiation factor 5	GDF5
108					
109	Mm.3514	NM_010834	Gdf8	Growth differentiation factor 8	GDF8
110	Mm.9714	NM_008110	Gdf9	Growth differentiation factor 9	GDF9
111	Mm.1239	NM_010277	Gfap	Glial fibrillary acidic protein	Gfap
112	Mm.298606	NM_008122	Gja7	Gap junction membrane channel protein alpha 7	Gja7
113	Mm.21198	NM_008124	Gjb1	Gap junction membrane channel protein beta 1	Gjb1
114	Mm.90003	NM_008126	Gjb3	Gap junction membrane channel protein beta 3	Gjb3
115	Mm.56906	NM_008127	Gjb4	Gap junction membrane channel protein beta 4	Gjb4
116	Mm.26859	NM_010291	Gjb5	Gap junction membrane channel protein beta 5	Gjb5
117	Mm.90364	NM_010493	Icam1	Intercellular adhesion molecule	ICAM-1
118	Mm.4629	NM_008319	Icam5	Intercellular adhesion molecule 5, telencephalon	ICAM-5
119	Mm.268521	NM_010512	Igf1	Insulinlike growth factor 1	IGF-1
120	Mm.275742	NM_010513	Igflr	Insulinlike growth factor I receptor	Igflr
121	Mm.3862	NM_010514	Igf2	Insulinlike growth factor 2	IGF-II
122	Mm.213226	NM_010515	Igf2r	Insulinlike growth factor 2 receptor	Igf2r
123	Mm.29254	NM_008343	Igfbp3	Insulinlike growth factor binding protein 3	Igfbp3
124	Mm.1019	NM_031168	Il6	Interleukin 6	IL-6

Gene Table (continued)

Position	Unigene	GeneBank	Symbol	Description	Gene name
125	Mm.2856	NM_010559	Il6ra	Interleukin 6 receptor, alpha	IL-6R
126	Mm.4364	NM_010560	Il6st	Interleukin 6 signal transducer	Gp130
127	Mm.276251	NM_146100	Ina	Internexin neuronal intermediate filament protein, alpha	Ina
128	Mm.8042	NM_008380	Inhba	Inhibin beta-A	INHBA
129	Mm.3092	XM_148966	Inhbb	Inhibin beta-B	INHBB
130	Mm.46269	NM_008386	Ins1	Insulin I	Insulin I
131	Mm.42041	NM_011832	Insrr	Insulin receptor-related receptor	Insrr
132	Mm.42242	NM_021459	Isl1	ISL1 transcription factor, LIM/homeodomain (islet 1)	Isl1
133	Mm.5007	NM_008396	Itga2	Integrin alpha 2	LFA1b/Cd49b
134	Mm.26646	NM_010575	Itga2b	Integrin alpha 2b	Cd41b
135	Mm.57035	NM_013565	Itga3	Integrin alpha 3	Cd49c
136	Mm.33596	NM_010576	Itga4	Integrin alpha 4	VLA-4
137	Mm.16234	NM_010577	Itga5	Integrin alpha 5 (fibronectin receptor alpha)	Integrin a5
138	Mm.225096	NM_008397	Itga6	Integrin alpha 6	Integrin a6
139	Mm.179747	NM_008398	Itga7	Integrin alpha 7	Integrin a7
140	Mm.329997	XM_140813	Itga8	Integrin alpha 8	Integrin a8
141	Mm.96	NM_008399	Itgae	Integrin, alpha E, epithelial-associated	Itgae
142	Mm.1618	NM_008400	Itgal	Integrin alpha L	LFA1a /CD11A
143	Mm.262106	NM_008401	Itgam	Integrin alpha M	Cd11b
144	Mm.227	NM_008402	Itgav	Integrin alpha V	Cd51
145	Mm.22378	NM_021334	Itgax	Integrin alpha X	Cd11c
146	Mm.263396	XM_134403	Itgb1	Integrin beta 1 (fibronectin receptor beta)	CD29
147	Mm.1137	NM_008404	Itgb2	Integrin beta 2	Cd18
148	Mm.87150	NM_016780	Itgb3	Integrin beta 3	CD61
149	Mm.213873	L04678	Itgb4	Integrin beta 4	Integrin b4
150	Mm.6424	NM_010580	Itgb5	Integrin beta 5	Integrin b5
151	Mm.98193	NM_021359	Itgb6	Integrin beta 6	Integrin b6
152	Mm.352620	NM_013566	Itgb7	Integrin beta 7	Integrin b7
153	Mm.294882	NM_172647	F11r	F11 receptor	Jcam1
154	Mm.285	NM_010612	Kdr	Kinase insert domain protein receptor	VEGFR2/FLK 1
155	Mm.6974	NM_016958	Krt1-14	Keratin complex 1, acidic, gene 14	k14
156	Mm.38498	NM_008469	Krt1-15	Keratin complex 1, acidic, gene 15	k15
157	Mm.14046	NM_010663	Krt1-17	Keratin complex 1, acidic, gene 17	Krt1-17
158	Mm.306829	XM_126758	Krt1-5	Keratin complex 1, acidic, gene 5	MHR a-1
159	Mm.29389	NM_031170	Krt2-8	Keratin complex 2, basic, gene 8	Krt2-8
160	Mm.4964	NM_008501	Lif	Leukemia inhibitory factor	LIF
161	Mm.149720	NM_013584	Lifr	Leukemia inhibitory factor receptor Lif	LifR
162	Mm.252063	NM_010777	Mbp	Myelin basic protein	Mbp
163	Mm.256966	NM_008632	Mtap2	Microtubule-associated protein 2	Mtab2
164	Mm.350936	NM_008634	Mtap1b	Microtubule-associated protein 1 B	Map5
165	Mm.250705	NM_013607	Myh11	Myosin heavy chain 11, smooth muscle	Myh11 (SM-MHC)
166	Mm.290003	NM_010856	Myh6	Myosin, heavy polypeptide 6, cardiac muscle, alpha	a-MHC
167	Mm.247636	NM_010858	Myl4	Myosin, light polypeptide 4, alkali; atrial, embryonic	MLC-1
168	Mm.4974	NM_010875	Ncam1	Neural cell adhesion molecule 1	NCAM
169	Mm.248541	NM_010954	Ncam2	Neural cell adhesion molecule 2	Ocam
170	Mm.23742	NM_016701	Nes	Nestin	Nes
171	Mm.266665	NM_010896	Neurog1	Neurogenin 1	Neurod3
172	Mm.1956	NM_010910	Nefl	Neurofilament, light polypeptide	Nfl
173	Mm.1259	NM_013609	Ngfb	Nerve growth factor, beta	NGF b
174	Mm.283893	NM_033217	Ngfr	Nerve growth factor receptor (TNFR superfamily, member 16)	Ngfr
175	Mm.4701	NM_010919	Nkx2-2	NK2 transcription factor related, locus 2 (<i>Drosophila</i>)	Nkx2-2
176	Mm.41974	NM_008700	Nkx2-5	NK2 transcription factor related, locus 5 (<i>Drosophila</i>)	Nkx2-5/Csx
177	Mm.57195	NM_013611	Nodal	Nodal	NODAL
178	Mm.39094	NM_008711	Nog	Noggin	NOG
179	Mm.290610	NM_008714	Notch1	Notch gene homolog 1 (<i>Drosophila</i>)	Notch1
180	Mm.254017	NM_010928	Notch2	Notch gene homolog 2 (<i>Drosophila</i>)	Notch2
181	Mm.4945	NM_008716	Notch3	Notch gene homolog 3 (<i>Drosophila</i>)	Notch3
182	Mm.173813	NM_010929	Notch4	Notch gene homolog 4 (<i>Drosophila</i>)	Notch4
183	Mm.254610	NM_011858	Odz4	Odd Oz/ten-m homolog 4 (<i>Drosophila</i>)	Odz4
184	Mm.6213	NM_008734	Nrg3	Neuregulin 3	NRG3
185	Mm.262663	NM_032002	Nrg4	Neuregulin 4	Nrg4
186	Mm.267570	NM_008742	Ntf3	Neurotrophin 3	Neurotrophin 3

(continued on next page)

Gene Table (continued)

Position	Unigene	GeneBank	Symbol	Description	Gene name
187	Mm.130054	NM_008745	Ntrk2	Neurotrophic tyrosine kinase, receptor, type 2	Ntrk2
188	Mm.33496	NM_008746	Ntrk3	Neurotrophic tyrosine kinase, receptor, type 3	Ntrk3
189	Mm.4390	NM_010949	Numb	Numb gene homolog (<i>Drosophila</i>)	Numb
190	Mm.39300	NM_016968	Olig1	Oligodendrocyte transcription factor 1	Olig1
191	Mm.37289	NM_016967	Olig2	Oligodendrocyte transcription factor 2	Olig2
192	Mm.3608	NM_013627	Pax6	Paired box gene 6	Pax6
193	Mm.2675	NM_008808	Pdgfa	Platelet-derived growth factor, alpha	PDGF a
194	Mm.144089	NM_011057	Pdgfb	Platelet-derived growth factor, B polypeptide	PDGF b
195	Mm.221403	NM_011058	Pdgfra	Platelet-derived growth factor receptor, alpha polypeptide	PDGFRa
196	Mm.4146	NM_008809	Pdgfrb	Platelet-derived growth factor receptor, beta polypeptide	PDGFRb
197	Mm.4949	NM_008814	Ipf1	Insulin promoter factor 1, homeodomain transcription factor	Pdx1
198	Mm.276652	NM_008816	Pecam	Platelet/endothelial cell adhesion molecule	PECAM1
199	Mm.1268	NM_011123	Plp	Proteolipid protein (myelin)	Plp
200	Mm.56945	NM_008899	Pou3f2	POU domain, class 3, transcription factor 2	Brn2
201	Mm.56944	NM_008900	Pou3f3	POU domain, class 3, transcription factor 3	Pou3f3 (brn-1)
202	Mm.17031	NM_013633	Pou5f1	POU domain, class 5, transcription factor 1	Pou5f1
203	Mm.28825	NM_010127	Pou6f1	factor 1	Brn5
204	Mm.25760	NM_011825	Prdc	Protein related to DAN and cerberus	Prdc
205	Mm.6250	NM_008935	Prom	Prominin 1	Prom
206	Mm.132579	NM_008937	Prox1	Prospero-related homeobox 1	Prox1
207	Mm.138472	NM_008957	Ptch	Patched homolog	Patched
208	Mm.287037	NM_008958	Ptch2	Patched homolog 2	Patched 2
209	Mm.245395	NM_008960	Pten	Phosphatase and tensin homolog	PTEN
210	Mm.143846	NM_011210	Ptprc	Protein tyrosine phosphatase, receptor type, C	Cd45
211	Mm.235998	NM_009115	S100b	S100 protein, beta polypeptide, neural	S100B
212	Mm.29580	NM_025285	Stmn2	Stathmin-like 2	SCG1
213	Mm.57202	NM_009170	Shh	Sonic hedgehog	Shh
214	Mm.267547	NM_011393	Slc1a2	Solute carrier family 1, member 2 glutamate transporter	MGLT1
215	Mm.6257	NM_009200	Slc1a6	Solute carrier family 1, member 6	GLT4
216	Mm.21002	NM_011400	Slc2a1	Solute carrier family 2 (facilitated glucose transporter), member 1	glucose transporter 1
217	Mm.2093	NM_011427	Snai1	Snail homolog 1 (<i>Drosophila</i>)	Sna
218	Mm.4272	NM_011415	Snai2	Snail homolog 2 (<i>Drosophila</i>)	slug
219	Mm.39088	NM_009233	Sox1	SRY-box containing gene 1	Sox1
220	Mm.276739	XM_128139	Sox10	SRY-box containing gene 10	Sox10
221	Mm.8575	NM_011439	Sox13	SRY-box containing gene 13	Sox13
222	Mm.347499	NM_009235	Sox15	SRY-box containing gene 15	Sox15
223	Mm.279103	NM_011441	Sox17	SRY-box containing gene 17	Sox17
224	Mm.264904	NM_009236	Sox18	SRY-box containing gene 18	Sox18
225	Mm.4541	NM_011443	Sox2	SRY-box containing gene 2	Sox2
226	Mm.35784	NM_009237	Sox3	SRY-box containing gene 3	Sox3
227	Mm.240627	NM_009238	Sox4	SRY-box containing gene 4	Sox4
228	Mm.1752	NM_011444	Sox5	SRY-box containing gene 5	Sox5
229	Mm.240042	NM_011445	Sox6	SRY-box containing gene 6	Sox6
230	Mm.286407	NM_011448	Sox9	SRY-box containing gene 9	Sox9
231	Mm.196611	NM_013680	Syn1	Synapsin I	Syn1
232	Mm.22421	NM_019766	Tebppending	Telomerase binding protein, p23	TEBP
233	Mm.152812	NM_009351	Tep1	Telomerase associated protein 1	Tep1
234	Mm.4306	NM_009352	Terf1	Telomeric repeat binding factor 1	Terf1
235	Mm.10109	NM_009354	Tert	Telomerase reverse transcriptase	Tert
236	Mm.248380	NM_011577	Tgfb1	Transforming growth factor, beta 1	TGFB1
237	Mm.18213	NM_009367	Tgfb2	Transforming growth factor, beta 2	TGF b2
238	Mm.307887	NM_009368	Tgfb3	Transforming growth factor, beta 3	TGF b3
239	Mm.197552	NM_009370	Tgfr1	Transforming growth factor, beta receptor I	ALK-5
240	Mm.172346	NM_009371	Tgfr2	Transforming growth factor, beta receptor II	TGFB2
241	Mm.200775	NM_011578	Tgfr3	Transforming growth factor, beta receptor III	Betaglycan
242	Mm.3951	NM_009382	Thy1	Thymus cell antigen 1, theta	Thy1
243	Mm.980	NM_011607	Tnc	Tenascin C	Tenascin C
244	Mm.40068	NM_023279	Tubb3	Tubulin, beta 3	Tubb3
245	Mm.10205	NM_009482	Utf1	Undifferentiated embryonic cell transcription factor 1	Utf1
246	Mm.76649	NM_011693	Vcam1	Vascular cell adhesion molecule 1	VCAM-1
247	Mm.282184	NM_009505	Vegfa	Vascular endothelial growth factor A	VEGF/VEGI
248	Mm.268000	NM_011701	Vim	Vimentin	Vim

Gene Table (continued)

Position	Unigene	GeneBank	Symbol	Description	Gene name
249	Mm.22182	NM_009519	Wnt11	Wingless-related MmTV Integration site 11	Wnt11
250	Mm.33653	NM_023653	Wnt2	Wingless-related MmTV Integration site 2	Wnt2
251	Mm.1367	NM_009522	Wnt3a	Wingless-related MmTV Integration site 3A	Wnt3a
252	Mm.20355	NM_009523	Wnt4	Wingless-related MmTV Integration site 4	Wnt4
253	Mm.22622	NM_009525	Wnt5b	Wingless-related MmTV Integration site 5B	Wnt5b
254	Mm.268282	NM_009526	Wnt6	Wingless-related MmTV Integrated site 6	Wnt6
255	Mm.306946	NM_009528	Wnt7b	Wingless-related MmTV Integration site 7B	Wnt7b
256	Mm.558	NM_009290	Wnt8a	Wingless-related MmTV Integration site 8A	Wnt8a
257	Mm.292297	NM_022981	Zfp110	Zinc finger protein 110	Zfp110
258	Mm.285848	NM_009556	Zfp42	Zinc finger protein 42	Zfp42
259					
260					
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263					
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266					
267					
268					
269	N/A	L08752	PUC18	PUC18 plasmid DNA	pUC18
270	N/A	L08752	PUC18	PUC18 plasmid DNA	pUC18
271	N/A	L08752	PUC18	PUC18 plasmid DNA	pUC18
272	N/A	L08752	PUC18	PUC18 plasmid DNA	pUC18
273	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
274	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
275	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
276	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
277	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
278	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
279	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
280	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
281	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
282	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
283	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
284	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
285	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin
286	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin
287	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin
288	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin

Appendix B. GEArray Q series mouse common cytokine gene array (Mm-003)

Array Layout

Agpt2 1	Aifl 2	Bmp1 3	Bmp2 4	Bmp4 5	Bmp5 6	Ppp1ca 7	Bmp7 8
Bmp8a 9	Csf1 10	Csf2 11	Csf3 12	Epo 13	Fgf1 14	Fgf10 15	Fgf11 16
Fgf12 17	Fgf14 18	Fgf16 19	Fgf17 20	Fgf18 21	Fgf2 22	Fgf20 23	Fgf21 24
Fgf22 25	Fgf3 26	Fgf4 27	Fgf5 28	Fgf6 29	Fgf7 30	Fgf9 31	Fgf32
Hgf 33	Ifna1 34	Ifna11 35	Ifna2 36	Ifna4 37	Ifna5 38	Ifna6 39	Ifna7 40
Ifna6 41	Ifnab 42	Ifnb 43	Ifng 44	Ifrd1 45	Igfl 46	Igf2 47	Il10 48
Il11 49	Il12a 50	Il12b 51	Il13 52	Il15 53	Il16 54	Il17 55	Il17b 56
Il18 57	Il1a 58	Il1b 59	Il2 60	Il20 61	Il3 62	Il4 63	Il5 64
Il6 65	Il7 66	Il9 67	Kitl 68	Lep 69	Lif 70	Lta 71	Ltb 72
Nfkbia 73	Pdgfa 74	Pdgfb 75	Ptn 76	Tgfa 77	Tgfb1 78	Tgfb2 79	Tgfb3 80
Thpo 81	Tnf 82	Tnfsf10 83	Tnfsf11 84	Tnfsf12 85	Tnfsf13b 86	Tnfsf14 87	Tnfsf4 88
Tnfsf5 89	Tnfsf6 90	Tnfsf7 91	Tnfsf8 92	Tnfsf9 93	Vegfa 94	Vegfb 95	Vegfc 96
PUC18 97	PUC18 98	PUC18 99	Blank 100	Blank 101	Blank 102	Gapd 103	Gapd 104
Ppia 105	Ppia 106	Ppia 107	Ppia 108	Rpl13a 109	Rpl13a 110	Actb 111	Actb 112

Gene Table

Position	Unigene	GeneBank	Symbol	Description	Gene name
1	Mm.3425	NM_007426	Agpt2	Angiopoietin 2	angiopoietin2
2	Mm.10747	NM_019467	Aif1	Allograft inflammatory factor 1	AIF1
3	Mm.27757	NM_009755	Bmp1	Bone morphogenetic protein 1	BmpP1
4	Mm.235230	NM_007553	Bmp2	Bone morphogenetic protein 2	BmpP2
5	Mm.6813	NM_007554	Bmp4	Bone morphogenetic protein 4	BmpP 4
6	Mm.118034	NM_007555	Bmp5	Bone morphogenetic protein 5	BmpP 5
7	Mm.1970	NM_031868	Ppp1ca	Protein phosphatase 1, catalytic subunit, alpha isoform	Ppp1ca
8	Mm.595	NM_007557	Bmp7	Bone morphogenetic protein 7	BmpP 7
9	Mm.270287	NM_007558	Bmp8a	Bone morphogenetic protein 8a	BmpP 8a, Oxct2a
10	Mm.795	NM_007778	Csf1	Colony stimulating factor 1 (macrophage)	M-CSF
11	Mm.4922	NM_009969	Csf2	Colony stimulating factor 2 (granulocyte-macrophage)	GM-CSF
12	Mm.1238	NM_009971	Csf3	Colony stimulating factor 3 (granulocyte)	G-CSF
13	Mm.349116	NM_007942	Epo	Erythropoietin	Epo
14	Mm.241282	NM_010197	Fgf1	Fibroblast growth factor 1	aFGF
15	Mm.317323	NM_008002	Fgf10	Fibroblast growth factor 10	FGF10
16	Mm.269011	NM_010198	Fgf11	Fibroblast growth factor 11	FGF11
17	Mm.7996	NM_010199	Fgf12	Fibroblast growth factor 12	FGF12A
18	Mm.32472	NM_010201	Fgf14	Fibroblast growth factor 14	FGF14 (FHF4)
19	Mm.154768	NM_030614	Fgf16	Fibroblast growth factor 16	FGF16
20	Mm.12814	NM_008004	Fgf17	Fibroblast growth factor 17	FGF17
21	Mm.246671	NM_008005	Fgf18	Fibroblast growth factor 18	FGF18
22	Mm.57094	NM_008006	Fgf2	Fibroblast growth factor 2	bFGF
23	Mm.348043	NM_030610	Fgf20	Fibroblast growth factor 20	FGF20
24	Mm.143736	NM_020013	Fgf21	Fibroblast growth factor 21	FGF21
25	Mm.154211	NM_023304	Fgf22	Fibroblast growth factor 22	FGF22
26	Mm.4947	NM_008007	Fgf3	Fibroblast growth factor 3	FGF3(int-2)
27	Mm.4956	NM_010202	Fgf4	Fibroblast growth factor 4	FGF4
28	Mm.5055	NM_010203	Fgf5	Fibroblast growth factor 5	FGF5
29	Mm.3403	XM_132863	Fgf6	Fibroblast growth factor 6	FGF6
30	Mm.330557	NM_008008	Fgf7	Fibroblast growth factor 7	FGF7/KGF
31	Mm.8846	NM_013518	Fgf9	Fibroblast growth factor 9	FGF9
32	Mm.297978	NM_010216	Figf	C-fos induced growth factor	VEGF-D/FIGF
33	Mm.267078	XM_131908	Hgf	Hepatocyte growth factor	HGF
34	Mm.57127	NM_010502	Ifna1	Interferon alpha family, gene 1	IFNA1
35	Mm.14102	NM_008333	Ifna11	Interferon alpha family, gene 11	IFN-a11
36	Mm.14091	NM_010503	Ifna2	Interferon alpha family, gene 2	IFNA2
37	Mm.57128	NM_010504	Ifna4	Interferon alpha family, gene 4	IFNA4
38	Mm.57129	NM_010505	Ifna5	Interferon alpha family, gene 5	IFNA5
39	Mm.6194	NM_008335	Ifna6	Interferon alpha family, gene 6	IFNA6
40	Mm.46795	NM_008334	Ifna7	Interferon alpha family, gene 7	IFNA7
41	Mm.6194	NM_008335	Ifna6	Interferon alpha family, gene 6	IFNA6
42	Mm.302572	NM_008336	Ifnab	Interferon alpha family, gene B	IFN a10
43	Mm.1245	NM_010510	Ifnb	Interferon beta, fibroblast	IFN-b1
44	Mm.240327	NM_008337	Ifng	Interferon gamma	IFN r
45	Mm.168	NM_013562	Ifrd1	Interferon-related developmental regulator 1	IFNRB1
46	Mm.268521	NM_010512	Igf1	Insulin-like growth factor 1	IGF-1
47	Mm.3862	NM_010514	Igf2	Insulin-like growth factor 2	IGF-II
48	Mm.874	NM_010548	Il10	Interleukin 10	IL-10
49	Mm.35814	NM_008350	Il11	Interleukin 11	IL-11
50	Mm.103783	NM_008351	Il12a	Interleukin 12A	IL-12A
51	Mm.239707	NM_008352	Il12b	Interleukin 12B	IL-12B
52	Mm.1284	NM_008355	Il13	Interleukin 13	IL-13
53	Mm.4392	NM_008357	Il15	Interleukin 15	IL-15
54	Mm.10137	NM_010551	Il16	Interleukin 16	IL-16
55	Mm.5419	NM_010552	Il17	Interleukin 17	IL-17
56	Mm.59313	NM_019508	Il17b	Interleukin 17B	IL17B
57	Mm.1410	NM_008360	Il18	Interleukin 18	IL-18
58	Mm.15534	NM_010554	Il1a	Interleukin 1 alpha	IL-1a
59	Mm.222830	NM_008361	Il1b	Interleukin 1 beta	IL-1b
60	Mm.14190	NM_008366	Il2	Interleukin 2	IL-2
61	Mm.103794	NM_021380	Il20	Interleukin 20	Il20

Gene Table (continued)

Position	Unigene	GeneBank	Symbol	Description	Gene name
62	Mm.983	NM_010556	Il3	Interleukin 3	IL-3
63	Mm.276360	NM_021283	Il4	Interleukin 4	IL-4
64	Mm.4461	NM_010558	Il5	Interleukin 5	IL-5
65	Mm.1019	NM_031168	Il6	Interleukin 6	IL-6
66	Mm.3825	NM_008371	Il7	Interleukin 7	IL-7
67	Mm.3006	NM_008373	Il9	Interleukin 9	IL-9
68	Mm.45124	NM_013598	Kitl	Kit ligand	SCF/MGF
69	Mm.277072	NM_008493	Lep	Leptin	ob
70	Mm.4964	NM_008501	Lif	Leukemia inhibitory factor	LIF
71	Mm.87787	NM_010735	Lta	Lymphotoxin A	TNFB
72	Mm.1715	NM_008518	Ltb	Lymphotoxin B	LT-b
73	Mm.170515	NM_010907	Nfkbia	Nuclear factor of kappa light chain gene enhancer in B-cells inhibitor, alpha	ikBa/Mad3
74	Mm.2675	NM_008808	Pdgfa	Platelet-derived growth factor, alpha	PDGF a
75	Mm.144089	NM_011057	Pdgfb	Platelet-derived growth factor, B polypeptide	PDGF b
76	Mm.279690	NM_008973	Ptn	Pleiotrophin	PTN
77	Mm.137222	NM_031199	Tgfa	Transforming growth factor alpha	TGF-a
78	Mm.248380	NM_011577	Tgfb1	Transforming growth factor, beta 1	TGFb1
79	Mm.18213	NM_009367	Tgfb2	Transforming growth factor, beta 2	TGF b2
80	Mm.307887	NM_009368	Tgfb3	Transforming growth factor, beta 3	TGF b3
81	Mm.3943	NM_009379	Thpo	Thrombopoietin	Thrombopoietin
82	Mm.1293	NM_013693	Tnf	Tumor necrosis factor	TNFa
83	Mm.1062	NM_009425	Tnfsf10	Tumor necrosis factor (ligand) superfamily, member 10	Trail
84	Mm.249221	NM_011613	Tnfsf11	Tumor necrosis factor (ligand) superfamily, member 11	TNFSF11
85	Mm.344820	NM_011614	Tnfsf12	Tumor necrosis factor (ligand) superfamily, member 12	APO3L
86	Mm.28835	NM_033622	Tnfsf13b	Tumor necrosis factor (ligand) superfamily, member 13b	TNFSFb
87	Mm.307668	NM_019418	Tnfsf14	Tumor necrosis factor (ligand) superfamily, member 14	HVEM-L
88	Mm.4994	NM_009452	Tnfsf4	Tumor necrosis factor (ligand) superfamily, member 4	OX40L
89	Mm.4861	NM_011616	Tnfsf5	Tumor necrosis factor (ligand) superfamily, member 5	CD40L
90	Mm.3355	NM_010177	Tnfsf6	Tumor necrosis factor (ligand) superfamily, member 6	FasL
91	Mm.42228	NM_011617	Tnfsf7	Tumor necrosis factor (ligand) superfamily, member 7	CD27L/CD70
92	Mm.4664	NM_009403	Tnfsf8	Tumor necrosis factor (ligand) superfamily, member 8	CD30L
93	Mm.41171	NM_009404	Tnfsf9	Tumor necrosis factor (ligand) superfamily, member 9	4-1BBL
94	Mm.282184	NM_009505	Vegfa	Vascular endothelial growth factor A	VEGF/VEGI
95	Mm.15607	NM_011697	Vegfb	Vascular endothelial growth factor B	VEGF-B
96	Mm.1402	NM_009506	Vegfc	Vascular endothelial growth factor C	VEGF-C
97	N/A	L08752	PUC18	PUC18 Plasmid DNA	pUC18
98	N/A	L08752	PUC18	PUC18 Plasmid DNA	pUC18
99	N/A	L08752	PUC18	PUC18 Plasmid DNA	pUC18
100	Blank	Blank	Blank	Blank	Blank
101	Blank	Blank	Blank	Blank	Blank
102	Blank	Blank	Blank	Blank	Blank
103	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
104	Mm.333399	NM_008084	Gapd	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH
105	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
106	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
107	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
108	Mm.5246	NM_008907	Ppia	Peptidylprolyl isomerase A	CyclophilinA
109	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
110	Mm.180458	NM_009438	Rpl13a	Ribosomal protein L13a	RPL13A
111	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin
112	Mm.297	NM_007393	Actb	Actin, beta, cytoplasmic	Beta-actin

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