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Characterization of tissue-specific *mbu-3* gene expression in the mouse central nervous system

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***Mbu-3* is a novel mouse brain unigene that was identified by digital differential display. In this study, expression of the gene was chased through developmental stages and the protein product was identified in the brain. The cDNA sequence was 3,995-bp long and contained an ORF of 745 AA. Database searches revealed that the chicken SST273 gene containing LRR- and Ig-domain was an *mbu-3* orthologue. Tissue specificity for the gene was examined in embryos and in brains at post-natal and adult stages. During the embryonic stages, *mbu-3* was localized to the central nervous system in the brain and spinal cord. In the early post-natal stages, the gene was evenly expressed in the brain. However, with aging, expression was confined to specific regions, particularly the hippocampus. The protein was approximately 95 kDa as determined by Western blot analysis of brain extracts. [BMB reports 2008; 41(12): 875-880]**

INTRODUCTION

By virtue of the mouse genome project and other large scale cDNA sequencing approaches, computer software tools are available to mine for tissue-specific genes or ESTs from the constructed databases (1-3). Over 90% of the information for the entire mouse genome was elucidated by the mouse genome project consortium and is now available in databases, such as Mouse Genome Resources (http://www.ncbi.nlm.nih.gov/projects/genome/guide/mouse/) and Mouse Ensembl (http://www.ensembl.org/Mus_musculus/) (4).

For cDNAs, large scale sequencing of mouse transcripts was carried out by a few groups with different experimental approaches. Approximately 9,500 mouse cDNAs were identified through the mammalian gene collection program (MGC) (http://mgc.nci.nih.gov) (5). In another study, the serial analysis of gene expression (SAGE) method identified groups of novel cDNAs in the mouse cerebellum and dorsal root ganglion (6, 7).

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Annotation of mouse cDNAs by *in situ* hybridization and GFP-fusion vector has also been performed. Extensive *in situ* hybridization analyses for brain sections were performed using genes expressed in the brain, and the results are available from the database (http://www.genepaint.org) (8). Cellular localization of genes was determined using a GFP-fused reporter system (9, 10). Gene expression profiles in embryonic and post-natal stages were analyzed using a GeneChip microarray (11).

The unigene is an organized view of the transcriptome (12, 13). For the mouse, 79,530 unigene entries are registered, which is the highest number available except for the 123,687 entries for human. Each unigene entry is a set of transcript sequences that appear to arise from the same transcription locus, along with information on protein similarities, gene expression, cDNA clone reagents and genomic location. Digital differential display (DDD) is an *in silico* approach by which tissue-specifically expressed or cancer-related genes can be quickly displayed from unigene databases (14-16). By analyzing differential gene expression patterns, it may be possible to identify genes that contribute to a cell's unique characteristics. The selected ESTs can then be used to obtain overlapping clones to construct full-length cDNA. Many novel genes, including nanog, cortexin-3 and net-1, were mined by DDD and analyzed further (17, 18).

Mbu-3 is a mouse brain-specific unigene mined in our previous study via the DDD program (19). In this study, gene structures, including full-length cDNA, exon/intron structure and AA sequence, were identified. Tissue specificity for the gene was initially addressed in the embryo and also for the brains of post-natal and adult mice. The gene was found to be exclusively expressed in the brain among various mouse tissues evaluated. This study may eventually offer important clues for elucidating the cellular function of the corresponding protein.

RESULTS

Identification of full-length *mbu-3* cDNA

In a previous study, we performed digital differential display to identify novel unigenes that showed tissue-specific expressions in the mouse brain; *mbu-3* was a candidate for which the structure and function have not been determined (19). The *mbu-3* EST showed the highest over-representation in the brain, but no expression in other tissues. In this study, the gene

structure of *mbu-3* was elucidated. Also, expression of the gene was chased in the developmental stages of the mouse, and the protein product was identified in the brain.

After the *mbu-3* unigene was initially screened, overlapping ESTs were collected and a cDNA clone, BC059068, was found to be the longest clone. BC059058 was identified by the Mammalian Gene Collection Program Team as a full-length mouse cDNA sequence that contained poly (A) at the 3'-end (5). To find clones that had extended 5'-UTR, we carried out 5'-RACE using a RACE-ready brain cDNA library. However, no clones obtained by RACE had a 5'-end that was the same or longer length than BC059068, implying that BC059068 was the full-length cDNA.

Mbu-3 (BC059068) is 3,995-bp long with an ORF of 2,235 bp. Alignment of the cDNA sequence with genomic DNA revealed that the gene spanned approximately 6 kb on mouse chromosome 9 and consisted of 3 exons. The 5'- and 3'-UTR sequences were 288- and 1,434-bp long, respectively, and the ORF encoded a 745-AA polypeptide (Fig. 1).

Characterization of the *mbu-3* gene

BLAST searches of the databases with the cDNA and protein sequences did not identify any mouse genes homologous to *mbu-3*. Only a chicken gene designated SST273 was shown to be a homologue with 52% AA identity. The SST273 gene was originally screened from a cDNA library of enriched chicken embryonic spinal motoneurons (20). The gene product is a member of the ISLR (immunoglobulin superfamily containing leucine-rich repeats). It is a transmembrane protein with 5 leucine-rich repeats (LRRs) and 1 immunoglobulin domain (21). SST273 is uniquely expressed in the embryonic spinal and cranial motoneurons at early developmental stages. However, no functional information in neurons is available.

These facts imply that *mbu-3* is the mouse homologue of chicken SST273, the functions of which have not yet been elucidated. When compared to SST273, *Mbu-3* showed relatively high homology with the LRR (67.5%) and transmembrane domain (68.2%) and low homology with the immunoglobulin domain (38.1%), which is longer in *Mbu-3* (124 AA) than in SST273 (97 AA) and contributes to the larger MW of *Mbu-3* (Fig. 2A).

Protein expression of *mbu-3* in brain tissue was confirmed by Western blot analysis of brain extracts. A peptide-EFEAGSEYSDRLPL-predicted to be located at the extracellular domain, was synthesized and injected into a rabbit to raise polyclonal antibodies. From the Western blot results, an approximately 95-kDa protein reacted with the antibody, although a few additional proteins of smaller MW also appeared with lower intensities (Fig. 3C). These results indicate that *mbu-3* is a novel gene expressed in the central nervous system of the mouse brain.

Tissue-specific expression of *mbu-3* in the central nervous system

Tissue specificity of *mbu-3* expression was examined by end-

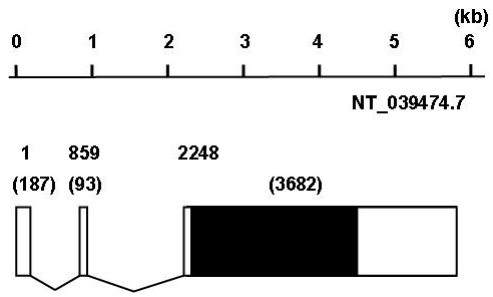

Fig. 1. Genomic organization of *mbu-3*. Genomic organization of the *mbu-3* gene is represented with exons (boxed) and introns (kinked horizontal lines) on the BAC clone, NT_039474.7. Exons are indicated at their relative locations (not drawn to scale). Open and black boxes indicate UTR and ORF, respectively. The exons are numbered starting from the first nucleotide of exon 1. Numbers in parentheses are the lengths of the exons in bp.

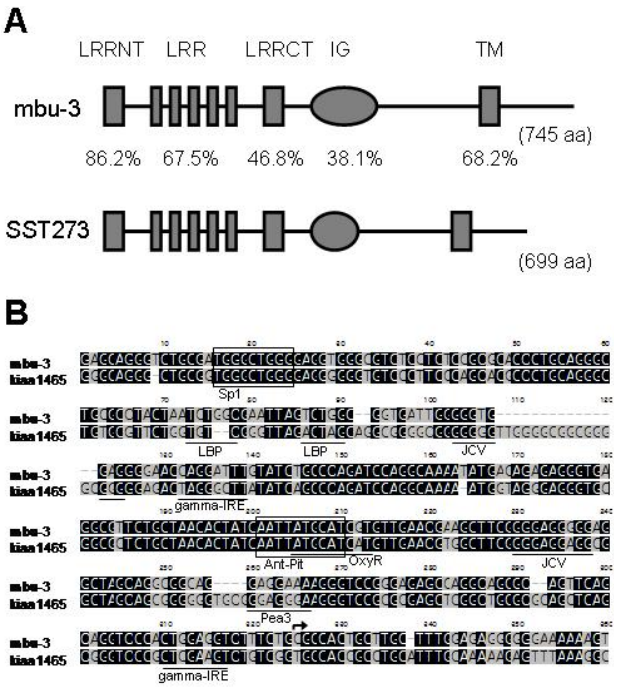

Fig. 2. Comparison of protein domain and DNA promoter regions of *mbu-3* with its homologues. (A) Schematic diagrams for domains of *mbu-3* and SST273 are depicted and homologies in each domain are indicated. LRRNT, leucine-rich repeat N-terminal; LRR, leucine-rich repeat; LRRCT, leucine-rich repeat C-terminal; IG, immunoglobulin; TM, transmembrane. (B) Alignment of upstream sequences of *mbu-3* and KIAA1465. Identical nucleotides in both sequences are shaded in black. Plausible transcription factor-binding sites for *mbu-3* are underlined with the name of the factor. Anterior-pit (Ant-Pit) and Sp1 binding sites appear in both species (opened box). The curved arrow marks the transcription start site.

point PCR using total RNA obtained from various mouse tissues. The results indicated that *mbu-3* was expressed exclusively in the brain (Fig. 3A). To monitor the expression of the gene in the brain in detail, real-time PCR was carried out for 8 brain tissues (Fig. 3B). Expression of the gene was found at high levels in the midbrain, cerebellum and posterior cerebral cortex. In the hypothalamus, thalamus, and hippocampus, moderate expression levels were detected. In the cerebrum, frontal cortex and hindbrain, relatively lower levels were observed.

To specifically localize the *mbu-3* transcript in brain tissue and in the embryo, *in situ* hybridization was carried out for sections of adult brain and embryos at a few different developmental stages (Fig. 4). In embryonic day 16 and 18 embryos, the *mbu-3* gene was expressed only in the brain and spinal cord, indicating that its expression is strictly confined to the central nervous system. At the post-natal and adult stages, the transcripts were evenly distributed throughout the brain and showed remarkable expression in the hippocampus. Control sections that were hybridized with the sense-strand probe did not show any specific localization.

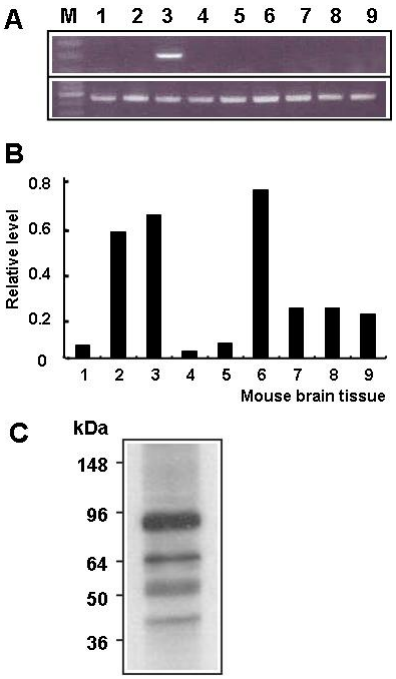

Fig. 3. Central nervous system-specific expression of *mbu-3*. (A) End-point RT-PCR of RNAs from various mouse tissues. Lanes are 1, heart; 2, kidney; 3, brain; 4, lung; 5, muscle; 6, spleen; 7, pancreas; 8, liver; 9, testis. M is a molecular weight marker. The figure at the bottom is the result for GAPDH as a control. (B) Tissue specificity and relative levels of *mbu-3* RNA in various brain tissues. Real-time PCR was performed and the expression level is indicated as the average of 3 independent reactions after the data had been normalized to GAPDH. Lanes are 1, cerebrum; 2, cerebellum; 3, frontal cortex; 4, posterior cortex; 5, hypothalamus; 6, hippocampus; 7, thalamus. (C) Identification of *Mbu-3* protein in mouse brain by Western blot analysis. 10 μg of mouse brain extract was loaded onto SDS-PAGE and immunoblotted with a polyclonal antibody raised against a synthetic peptide (EFEAGSEYSDRLPL) deduced from the *Mbu-3* amino acid sequence.

DISCUSSION

In this study, we characterized the structure and expression specificity of a novel mouse gene, *mbu-3*, which was exclusively expressed in the central nervous system. A BLAST search found no homologous mouse genes, implying that *mbu-3* is a novel gene. A chicken homologue, SST273, was found with 52% AA identity. We found that expression of *mbu-3* is confined to the brain and spinal cord. The expression profile of the *mbu-3* unigene conferred by the NCBI database (http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer) also indicated the strict tissue specificity of the gene. These facts indicate that these 2 homologous genes might be similarly regulated at the promoter to elicit neuron-specific expressions in both the mouse and chicken.

At present, no information regarding the promoter activity of *mbu-3* or its homologues is available. In the upstream region of *mbu-3* and its human homologue KIAA1465, no TATA-like sequence was found. No sequence data are currently available from the database for chicken SST273. A few transcription factor-binding motifs were identified in the upstream sequence of

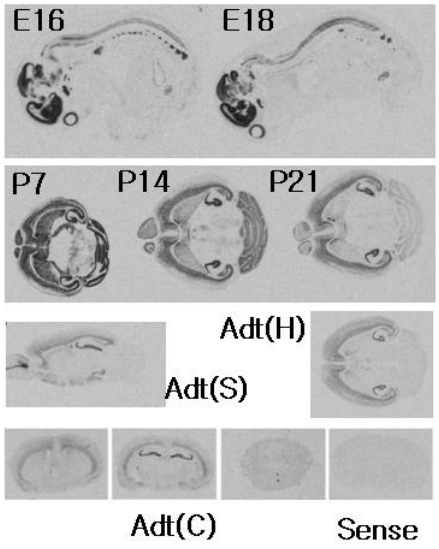

Fig. 4. Localization of *mbu-3* RNA in mouse embryo and brain. The ^{35}S-labeled RNA probe was *in situ* hybridized to embryo and brain sections. Sections E16 and E18 are embryos on day 16 and 18, respectively. P7, P14, and P21 represent postnatal day 7, 14, and 21, respectively. Adt (H), Adt (S), and Adt (C) indicate horizontal, sagittal, and coronal sections of adult brains, respectively. Sense-strand probe was used as a negative control (Sense).

mbu-3. When *mbu-3* and KIAA1465 sequences were aligned, they showed 52% sequence identity over the 324 bp that were compared. Binding sites for Sp1 and Anterior-Pit were common to both species. To identify any cis-elements upstream of the gene, we constructed recombinant luciferase reporter vectors containing variable lengths of the upstream sequences and examined their promoter activity after transfecting the DNAs into NG108-15 and HEK293 cells, which have proven to work well for other brain-specific genes in our previous studies (19, 22). Less than two-fold promoter activity, compared to the basic promoter element, was induced by the 2-kb upstream sequences in both cell types. This result may indicate that the 2 kb is not sufficient for promoter activity; otherwise, these cell lines may not accommodate the gene with a suitable environment for expression.

Additional screening of positive-acting elements in other neuronal cell cultures and elucidation of the relationship between the elements and tissue specificity should provide useful information about the properties of these elements.

Brain-specific expression of the *mbu-3* gene was confirmed through multiple methods: end-point and real-time RT-PCR, *in silico* gene expression profiling and *in situ* hybridization analysis of tissue sections. These methods revealed similar tissue specificity for the gene. For example, higher expressions in the cerebellum, midbrain and posterior cerebral cortex, as well as in the brain and spinal cord, compared to other tissues were well-matched in the RT-PCR and *in situ* hybridization analysis. These results were also very similar to the expression profile suggested by *in silico* analysis of EST counts. It is interesting that *mbu-3* expression was focused in the hippocampus in the *in situ* hybridization results. The gene was vividly and evenly expressed in the brain at P7 (seventh postnatal day). Then, as the mouse aged, expression was restricted to specific regions, especially to the hippocampus. In this respect, the *mbu-3* gene could be used to study gene activities in the hippocampus, which is a pivotal area for learning and memory.

Considering all these results, we conclude that *mbu-3* is a novel mouse gene, the expression of which is restricted to the central nervous system. After it was mined from UniGene databases by *in silico* DDD analysis, the gene was proven to be real and its expression profile was elucidated as being nervous system-specific. Further research, such as identification of cis-acting and trans-acting elements responsible for the neuronal cell-specific expression and knock-down or knock-out of the gene, will aid in elucidating the function of *mbu-3* in the nervous system.

MATERIALS AND METHODS

Sequence analysis

Mbu-3 was one of the ESTs that were over-represented in brain tissue-derived libraries after carrying out digital differential display (http://www.ncbi.nlm.nih.gov/UniGene/info_ddd.shtml) (19). ESTs overlapping with *mbu-3* were located using the general BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). cDNA fragments extended by RACE from the EST were subcloned into the pGEM-T vector (Promega, Madison, WI) and sequenced. The genomic sequence of *mbu-3* was identified using mouse BLAST (http://www.ncbi.nlm.nih.gov/genome/seq/MmBlast.html). ORF and amino acid sequences were deduced using an ORF finding program (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and DNASIS.MAX version 2.0 for Windows (Miraibio, Alameda, CA).

End-point RT-PCR

Total RNA of ICR mouse tissue was prepared with Trizol reagent according to the manufacturer's protocol (GibcoBRL, Carlsbad, CA). Reverse transcription was carried out using 10 μg of total RNA and a reverse transcription kit (Promega, Madison, WI). Tissue specificity was determined by nested PCR using primers deduced from the cDNA sequence of *mbu-3*. The primers - 5'-CCTGGAGAAGTAGGGCGAAC and 5'-GCAAAGCCCAGGCCAAACA for the primary PCR and 5'-GGACTTTGGGGCCAAGGGGA and 5'-GCTCCAAAGGGCCCCATCG for the secondary PCR- were used to amplify a 179-bp fragment. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were used to normalize *mbu-3* expression; their sequences were 5'-ACCACAGTCCATGCCATCAC and 5'-TCCACCACCCTGTTGCTGTA. A total of 35 cycles of PCR were performed; each cycle consisted of 94°C for 45 seconds, 62°C for 1 minute, and then 72°C for 40 seconds.

Quantitative real-time RT-PCR

Reverse transcription was performed as described above for end-point RT-PCR. PCR reactions were carried out as described previously (22). The partial cycle resulting in a statistically significant increase in the *mbu-3* product was determined and normalized to GAPDH. The probe sequence used for *mbu-3* was 5'-CAGCGACATTTCTGGGTCTGGTGCT.

Western blot analysis

To raise a polyclonal antibody against the *MBU-3* protein, a peptide (EFEAGSEYSDRLPL), the sequence of which corresponded to the carboxyl-end of *Mbu-3*, was chemically synthesized (Peptron, Korea) and subcutaneously injected into a rabbit. Blood was collected 6 weeks after antigen injection. Serum was diluted 1:1,000 and used for immunoblotting. Mouse brain extract was obtained by homogenizing a mouse brain in 500 μl of Pro-Prep lysis buffer (Intron, Korea) and centrifuged at 12,000×g for 10 min; 30 μg of total protein from the supernatant was loaded onto 8% SDS-PAGE.

In situ hybridization

Expression of the *mbu-3* transcript was examined by *in situ* hybridization of embryo and brain sections of ICR mice as previously described (23). Twelve-μm thick sections were prepared by cryostat. To prepare a riboprobe, a 558-bp DNA fragment of the *mbu-3* cDNA (nucleotides 262-773) was subcl-

oned into the pGEM-T vector (Promega, Madison, WI) and *in vitro*-transcribed using T7 and SP6 RNA polymerase in the presence of α-[^{35}S]UTP (1,000-1,500 Ci/mmol, Amersham, Piscataway, NJ). The probe RNA was hybridized with tissue sections overnight at 53°C. After hybridization, slides were washed with 0.2×SSC at 60°C for 60 min and exposed to β-max film (Amersham).

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REFERENCES

1. Pao, S. Y., Lin, W. L. and Hwang, M. J. (2006) In silico identification and comparative analysis of differentially expressed genes in human and mouse tissues. *BMC Genomics* **7**, 86.
2. Cahoy, J. D., Emery, B., Kaushal, A., Foo, L. C., Zamanian, J. L., Christopherson, K. S., Xing, Y., Lubischer, J. L., Krieg, P. A., Krupenko, S. A., Thompson, W. J. and Barres, B. A. (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J. Neurosci*. **28**, 264-278.
3. Gofflot, F., Chartoire, N., Vasseur, L., Heikkinen, S., Dembele, D., Le Merrer, J. and Auwerx, J. (2007) Systematic gene expression mapping clusters nuclear receptors according to their function in the brain. *Cell* **131**, 405-418.
4. Flicek, P., Aken, B. L., Beal, K., Ballester, B., Caccamo, M., Chen, Y., Clarke, L., Coates, G., Cunningham, F., Cutts, T., Down, T., Dyer, S. C., Eyre, T., Fitzgerald, S., Fernandez-Banet, J., Gräf, S., Haider, S., Hammond., M., Holland, R., Howe, K.L., Howe, K., Johnson, N., Jenkinson, A., Kähäri, A., Keefe, D., Kokocinski, F., Kulesha E., Lawson, D., Longden, I., Megy, K., Meidl, P., Overduin, B., Parker, A., Pritchard, B., Prlic, A., Rice, S., Rios, D., Schuster, M., Sealy, I., Slater, G., Smedley, D., Spudich, G., Trevanion, S., Vilella, A.J., Vogel, J., White, S., Wood, M., Birney, E., Cox, T., Curwen V., Durbin, R., Fernandez-Suarez, X. M., Herrero, J., Hubbard, T. J., Kasprzyk, A., Proctor, G., Smith, J., Ureta-Vidal, A., Searle, S. (2008) Ensembl 2008. *Nucleic Acids Res*. **36**, D707-714.
5. Strausberg, R. L., Feingold, E. A., Grouse, L. H., Derge, J. G., Klausner, R. D., Collins, F. S., Wagner, L., Shenmen, C. M., Schuler, G. D., Altschul, S. F., Zeeberg, B., Buetow, K. H., Schaefer, C. F., Bhat, N. K., Hopkins, R. F., Jordan, H., Moore, T., Max, S. I., Wang, J., Hsieh, F., Diatchenko, L., Marusina, K., Farmer, A. A., Rubin, G. M., Hong, L., Stapleton, M., Soares, M. B., Bonaldo, M. F., Casavant, T. L., Scheetz, T. E., Brownstein, M. J., Usdin, TB., Toshiyuki, S., Carninci, P., Prange, C., Raha, S. S., Loquellano, N. A., Peters, G. J., Abramson, R. D., Mullahy, S. J., Bosak, S. A., McEwan, P. J., McKernan, K. J., Malek, J. A., Gunaratne, P. H., Richards, S., Worley, K. C., Hale, S., Garcia, A. M., Gay, L. J., Hulyk, S. W., Villalon, D. K., Muzny, D. M., Sodergren, E. J., Lu, X., Gibbs, R. A., Fahey, J., Helton, E., Ketteman, M., Madan, A., Rodrigues, S., Sanchez, A., Whiting, M., Madan, A., Young, A. C., Shevchenko, Y., Bouffard, G. G., Blakesley, R. W., Touchman, J. W., Green, E. D., Dickson, M. C., Rodriguez, A. C., Grimwood, J., Schmutz, J., Myers, R. M., Butterfield, Y. S., Krzywinski, M. I., Skalska, U., Smailus, D. E., Schnerch, A., Schein, J. E., Jones, S. J., Marra, M. A. (2002) Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc. Natl. Acad. Sci. U. S. A*. **99**, 16899-16903.
6. Popesco, M. C., Frostholm, A., Rejniak, K. and Rotter, A. (2004) Digital transcriptome analysis in the aging cerebellum. *Ann. N. Y. Acad. Sci*. **1019**, 58-63.
7. Bourane, S., Mechaly, I., Venteo, S., Garces, A., Fichard, A., Valmier, J. and Carroll, P. (2007) A SAGE-based screen for genes expressed in sub-populations of neurons in the mouse dorsal root ganglion. *BMC Neurosci*. **8**, 97.
8. Jo, M., Gieske, M. C., Payne, C. E., Wheeler-Price, S. E., Gieske, J. B., Ignatius, I. V., Curry, T. E., Jr. and Ko, C. (2004) Development and application of a rat ovarian gene expression database. *Endocrinology* **145**, 5384-5396.
9. Fujii, G., Tsuchiya, R., Ezoe, E. and Hirohashi, S. (1999) Analysis of nuclear localization signals using a green fluorescent protein-fusion protein library. *Exp. Cell Res*. **251**, 299-306.
10. Alting-Mees, M. A., Risseeuw, E. P., Liu, E., Desautels, M., Crosby, W. A. and Hemmingsen, S. M. (2006) Intracellular expression of recombinant antibody fluorescent protein fusions for localization of target antigens in Schizosaccharomyces pombe. Methods *Mol. Biol*. **313**, 97-105.
11. Matsuki, T., Hori, G. and Furuichi, T. (2005) Gene expression profiling during the embryonic development of mouse brain using an oligonucleotide-based microarray system. *Brain Res. Mol. Brain Res*. **136**, 231-254.
12. Stanton, J. A., Macgregor, A. B., Mason, C., Dameh, M. and Green, D. P. (2007) Building comparative gene expression databases for the mouse preimplantation embryo using a pipeline approach to UniGene. *Mol. Hum. Reprod*. **13**, 713-720.
13. Brandenberger, R., Wei, H., Zhang, S., Lei, S., Murage, J., Fisk, G. J., Li, Y., Xu, C., Fang, R., Guegler, K., Rao, M. S., Mandalam, R., Lebkowski, J. and Stanton, L. W. (2004) Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nat. Biotechnol*. **22**, 707-716.
14. Tokuzawa, Y., Maruyama, M. and Yamanaka, S. (2006) Utilization of digital differential display to identify novel targets of Oct3/4. *Methods Mol. Biol*. **329**, 223-231.
15. Leyden, J., Murray, D., Moss, A., Arumuguma, M., Doyle, E., McEntee, G., O'Keane, C., Doran, P. and MacMathuna, P. (2006) Net1 and Myeov: computationally identified mediators of gastric cancer. *Br. J. Cancer* **94**, 1204-1212.
16. Lv, D., Song, P., Chen, Y., Gong, W. and Mo, S. (2005) Cloning and characterization of full length of a novel zebrafish gene Zsrg abundantly expressed in the germline stem cells. *Biochem. Biophys. Res. Commun*. **329**, 632-637.
17. Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M. and Yamanaka, S. (2003) The homeoprotein Nanog is required

for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113**, 631-642.
18. Wang, H. T., Chang, J. W., Guo, Z. and Li, B. G. (2007) In silico-initiated cloning and molecular characterization of cortexin 3, a novel human gene specifically expressed in the kidney and brain, and well conserved in vertebrates. *Int. J. Mol. Med.* **20**, 501-510.
19. Yang, H. L., Cho, E. Y., Han, K. H., Kim, H. and Kim, S. J. (2007) Characterization of a novel mouse brain gene (mbu-1) identified by digital differential display. *Gene* **395**, 144-150.
20. Gejima, R., Okafuji, T. and Tanaka, H. (2006) The LRR and Ig domain-containing membrane protein SST273 is expressed on motoneurons. *Gene Expr. Patterns* **6**, 235-240.
21. Nagasawa, A., Kubota, R., Imamura, Y., Nagamine, K., Wang, Y., Asakawa, S., Kudoh, J., Minoshima, S., Mashima, Y., Oguchi, Y. and Shimizu, N. (1997) Cloning of the cDNA for a new member of the immunoglobulin superfamily (ISLR) containing leucine-rich repeat (LRR). *Genomics* **44**, 273-279.
22. Ji, J. W., Yang, H. L. and Kim, S. J. (2006) Analysis of cag-8: a novel poly (Q) -encoding gene in the mouse brain. *Biochem. Biophys. Res. Commun.* **346**, 1254-1260.
23. Kim, Y., Kim, E. H., Hong, S., Rhyu, I. J., Choe, J., Sun, W. and Kim, H. (2006) Expression of thymosin beta in the rat brain following transient global ischemia. *Brain Res.* **1085**, 177-182.