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Ko et al.

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(54) **METHODS FOR MODULATING EMBRYONIC STEM CELL DIFFERENTIATION**

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(73) Assignee: **The United States of America as represented by the Secretary of the Department of Health and Human Services**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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Related U.S. Application Data

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(51) **Int. Cl.**

C12Q 1/68	(2006.01)
C12N 15/85	(2006.01)
C12N 5/10	(2006.01)

(52) **U.S. Cl.**
USPC **435/6.1**; 435/320.1; 435/325

(58) **Field of Classification Search**
USPC 435/6.1, 320.1, 325
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,226,914	A	7/1993	Caplan et al.	
5,670,372	A	9/1997	Hogan	
5,750,376	A	5/1998	Weiss et al.	
6,090,622	A	7/2000	Gearhart et al.	
6,200,806	B1	3/2001	Thomson	
6,506,559	B1	1/2003	Fire et al.	
6,943,241	B2	9/2005	Isogai et al.	
2002/0127715	A1 *	9/2002	Benvenisty et al.	435/366
2006/0251642	A1	11/2006	Wolffe et al.	

FOREIGN PATENT DOCUMENTS

JP	09-500004	1/1997
WO	WO 94/24274	10/1994
WO	WO 00/27995	5/2000
WO	WO 00/70021	11/2000
WO	WO/2004/009768 *	1/2004
WO	WO 2004/067744	8/2004

OTHER PUBLICATIONS

Romano, Drug News Prospect, 2003, 16(5): 267, 267-276.*
Andrews et al Biochem Soc Trans. 2005; 33(Pt 6):1526-30.*
Koestenbauer et al Am J Reprod Immunol. 2006; 55(3):169-80.*
Zhang et al. (Nucleic Acid Research, 2006, 34(17), 4780-4790.*
Falco et al (PowerPoint presentation from the Annual Meeting of the Society for the Study of Reproduction, Jul. 24-27, 2005) or Abstract from the Annual Meeting of the Society for the Study of Reproduction.*
International Search Report from PCT/US2008/058261, dated Jan. 19, 2009.
Written Opinion of the International Searching Authority from PCT/US2008/058261, dated, Jan. 19, 2009.
Andrews et al., "Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: opposite sides of the same coin," *Biochem Soc Trans* 33(Pt6):1526-1530, 2005.
Database Geneseq [Online], "Viral vector-related plasmid—pcDNA6.2/GFP-DEST." XP002492234 retrieved from EBI accession No. GSN:ADQ48564 Database accession No. ADQ48564, Sep. 9, 2004.
Carter et al., "An in situ hybridization-based screen for heterogeneously expressed genes in mouse ES cells," *Gene Expression Patterns* 8(3):181-198, Nov. 4, 2007.
Chambers et al., "Functional Expression Cloning of Nanog, a Pluripotency Sustaining Factor in Embryonic Stem Cells," *Cell*113:643-655, 2003.
Dahéron et al., "LIF/STAT3 Signaling Fails to Maintain Self-Renewal of Human Embryonic Stem Cells," *Stem Cells* 22:770-778, 2004.
Edelstein et al., "The SCAN domain family of zinc finger transcription factors," *Gene* 359:1-1, Oct. 10, 2005.
Falco, "*Zga1*, a 2-cell Specific Gene Required for 2-cell to 4-cell Progression in Mouse Preimplantation Embryos," PowerPoint presentation from the Annual Meeting of the Society for the Study of Reproduction, Jul. 24-27, 2005.
Falco et al., "Identification and Characterization of Zygotic Genomic Activation Gene 1 (Zga1) in Mouse," Abstract from the Annual Meeting of the Society for the Study of Reproduction, Jul. 24-27, 2005.
Falco et al., "Zscan4: A novel gene expressed exclusively in late 2-cell embryos and embryonic stem cells," *Developmental Biology* 307(2):539-550, 2007.

(Continued)

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(74) *Attorney, Agent, or Firm* — Klarquist Sparkman, LLP

(57) **ABSTRACT**

Described herein is Zscan4, a gene exhibiting 2-cell embryonic stage and embryonic stem cell specific expression. Identification of nine Zscan4 co-expressed genes is also described. Inhibition of Zscan4 expression inhibits the 2-cell to 4-cell embryonic transition and prevents blastocyst implantation, expansion and outgrowth. Provided herein are methods of inhibiting differentiation of a stem cell, promoting blastocyst outgrowth of embryonic stem cells and identifying a subpopulation of stem cells expressing Zscan4. Further described is the identification of Trim43 as a gene exhibiting morula-specific expression. Also provided are isolated expression vectors comprising a Zscan4 promoter, or a Trim43 promoter operably linked to a heterologous polypeptide and uses thereof. Further provided are transgenic animals comprising transgenes encoding marker proteins operably linked to Zscan4 and Trim43 promoters.

7 Claims, 16 Drawing Sheets

(56) **References Cited**

OTHER PUBLICATIONS

Falco et al., "Use of *Chuk* as an internal standard suitable for quantitative RT-PCR in mouse preimplantation embryos," *Reprod Biomed Online* 13(3):397-403, 2006.

Gerhard et al., The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC), *Genom Res 14*:2121-2127, 2004.

Ginis et al., "Differences between human and mouse embryonic stem cells," *Dev Biol* 269:360-380, 2004.

Humphrey et al., "Maintenance of Pluripotency in Human Embryonic Stem Cells is STAT3 Independent," *Stem Cells* 22:522-530, 2004.

Koestenbauer et al., "Embryonic Stem Cells: Similarities and Differences Between Human and Murine Embryonic Stem Cells," *Am J Reprod Immunol* 55(3):169-180, 2006.

Ota et al., Complete sequencing and characterization of 21,243 full-length human cDNAs, *Nat Genet* 36(1):40-45, 2004.

Romano, "Gene Transfer in Experimental Medicine," *Drug News Prospect* 16(5):267-276, 2003.

Sato et al., "Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor," *Nat Med* 10:55-63, 2004.

Sharov et al., Transcriptome analysis of mouse stem cells and early embryos, *PLoS Bio* 1(3):410-419, 2003.

Strausberg et al., Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences, *Proc Natl Acad Sci USA* 99(26):16899-16903, 2002.

Thomas et al., "Progress and Problems with the Use of Viral Vectors for Gene Therapy," *Nature Rev Genet* 4:346-358, 2004.

Verma and Weitzman, "Gene Therapy: Twenty-First Century Medicine," *Annu Rev Biochem* 74:711-738, 2005.

Zhang et al., Zfp206 regulates ES cell gene expression and differentiation, *Nucleic Acids Research* 34(17):4780-4790, 2006.

Mitsui et al., "The Homeoprotein Nanog is Required for Maintenance of Pluripotency in Mouse Epiblast and ES Cells," *Cell* 113:631-642, 2003.

* cited by examiner

FIG. 1A

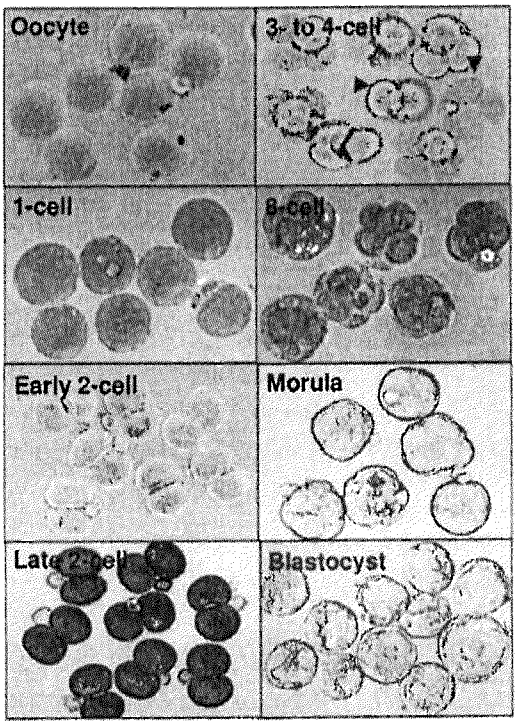

Oocyte
3- to 4-cell
1-cell
8-cell
Early 2-cell
Morula
Late 2-cell
Blastocyst

FIG. 1B

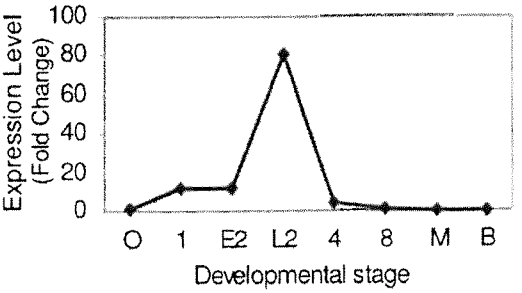

Expression Level (Fold Change)
100
80
60
40
20
0
O
1
E2
L2
4
8
M
B
Developmental stage

FIG. 2A

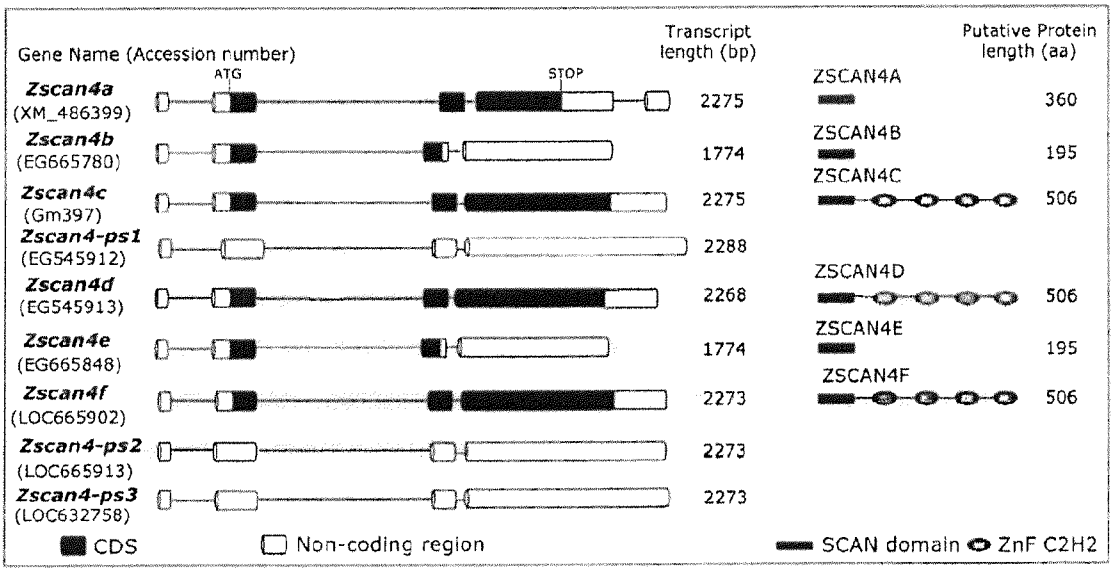
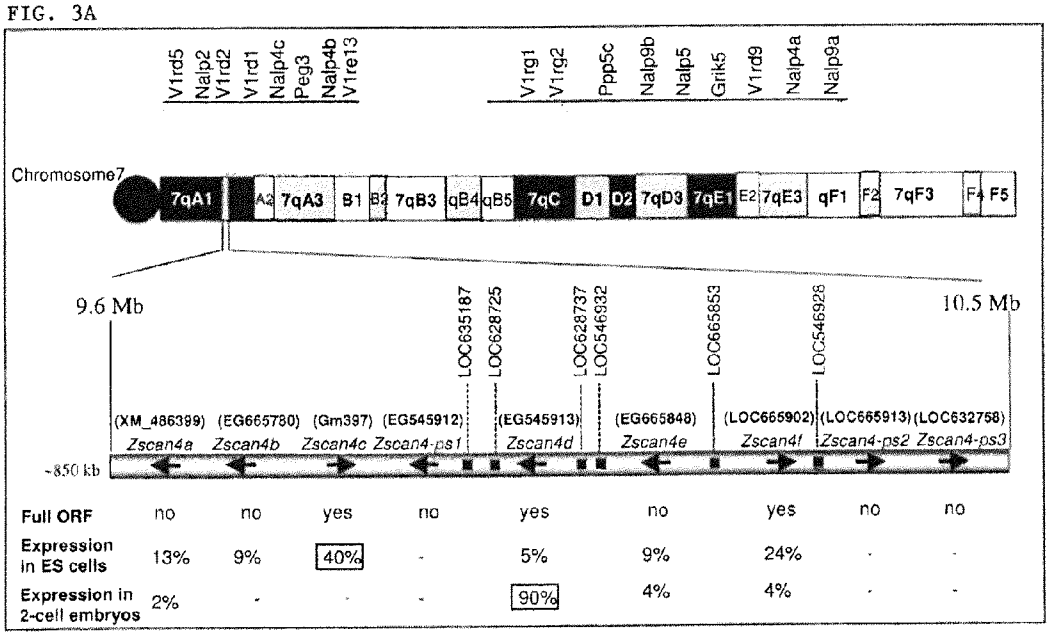
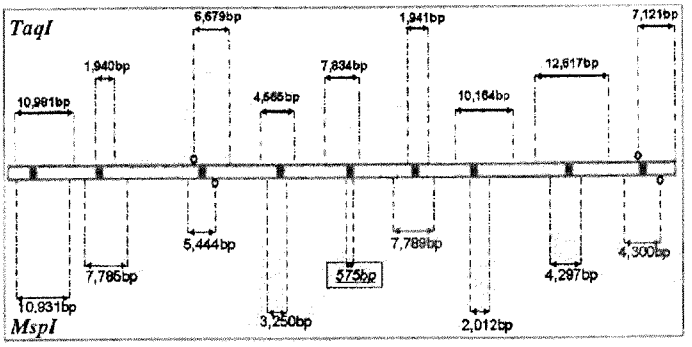

Gene Name (Accession number)
ATG
STOP
Transcript length (bp)
Zscan4a (XM_486399)
2275
Zscan4b (EG665780)
1774
Zscan4c (Gm397)
2275
Zscan4-ps1 (EG545912)
2288
Zscan4d (EG545913)
2268
Zscan4e (EG665848)
1774
Zscan4f (LOC665902)
2273
Zscan4-ps2 (LOC665913)
2273
Zscan4-ps3 (LOC632758)
2273
CDS
Non-coding region

FIG. 2B

Putative Protein length (aa)
ZSCAN4A
360
ZSCAN4B
195
ZSCAN4C
506
ZSCAN4D
506
ZSCAN4E
195
ZSCAN4F
506
SCAN domain
ZnF C2H2

FIG. 3A





♦=Target sequences of the probe used for Southern Blot hybridization
o= Restriction sites that generate extra bands in double digestion with *MspI/TaqI*

FIG. 3B

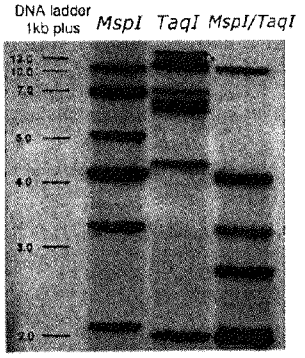


FIG. 3C

FIG. 4A

Name of siRNA	Target positions on cDNA (bp)	Target sequences
Plus-siZscan4 (J-064700-05: Dharmacon)	514-532 (exon II)	gtagcgatatgaggagatt
Plus-siZscan4 (J-064700-06: Dharmacon)	236-254 (exon II)	gaccaacaatttagagttt
Plus-siZscan4 (J-064700-07: Dharmacon)	304-322 (exon II)	caccaagtgctcagctaaa
Plus-siZscan4 (J-064700-08: Dharmacon)	362-380 (exon II)	gctgcaaagtctctggaag
siZscan4 (Zscan4_stealth508: Invitrogen)	508-532 (exon II)	ccagtggtagcgatatgaggagatt
shZscan4 (Genscript)	1463-1481 (exon IV)	gagtgaattgctttgtgtc

FIG. 4B

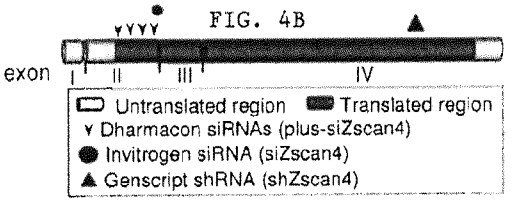


FIG. 4C

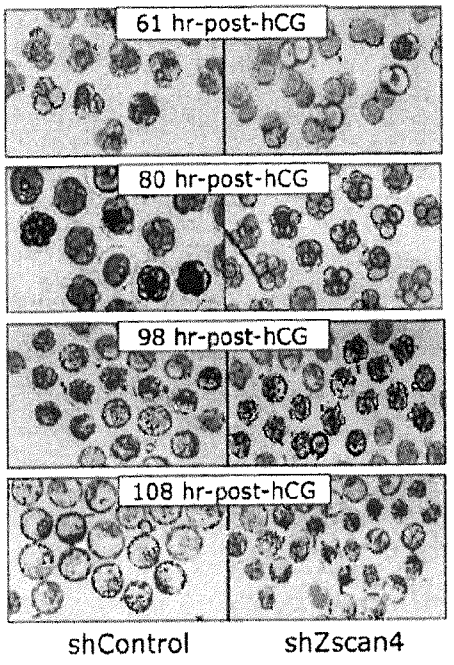
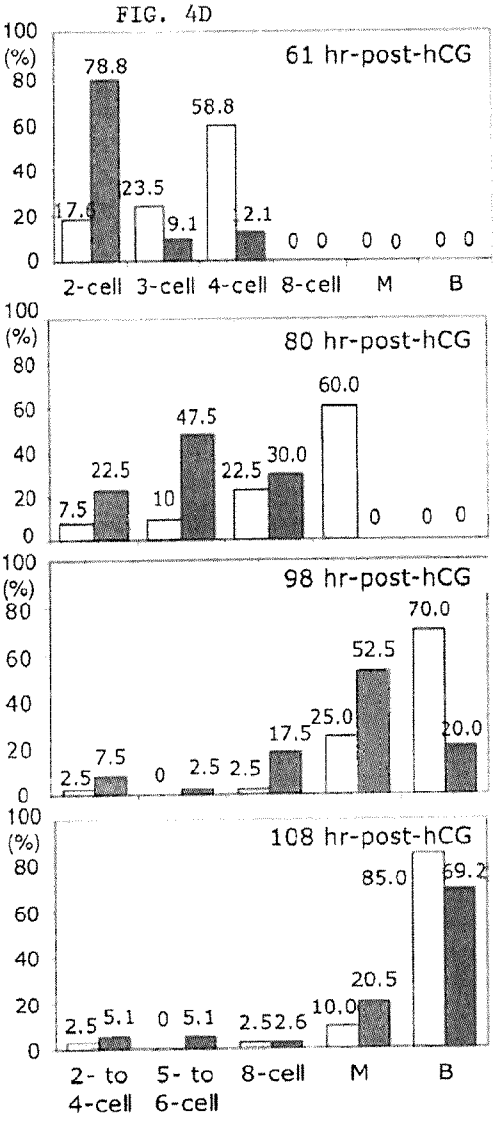


FIG. 4D



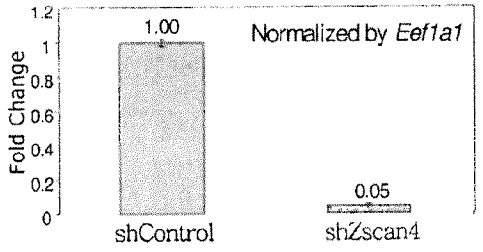


FIG. 4E

FIG. 5A

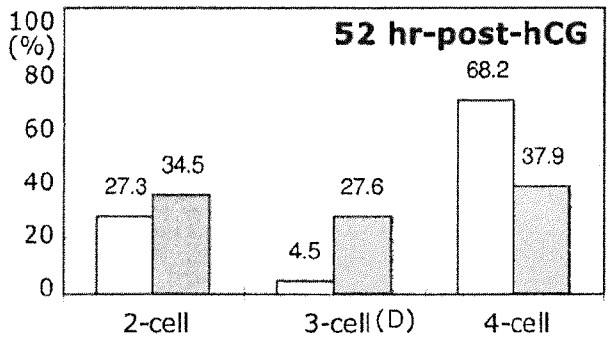


FIG. 5B

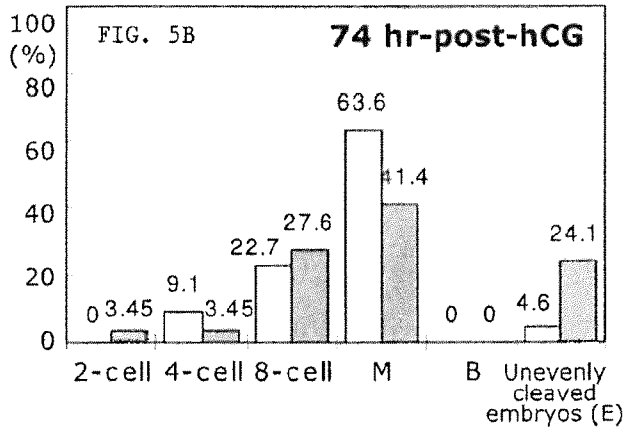


FIG. 5C

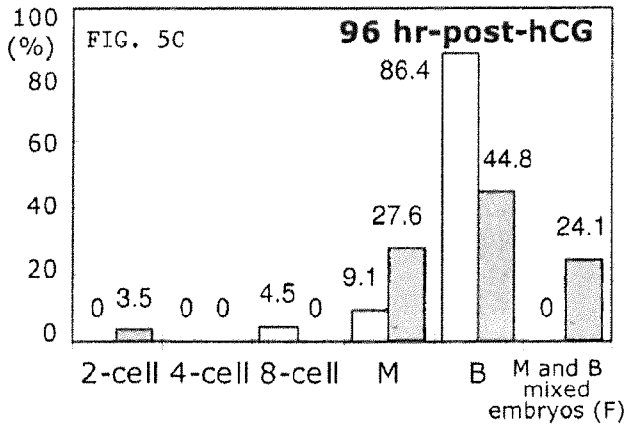


FIG. 5D

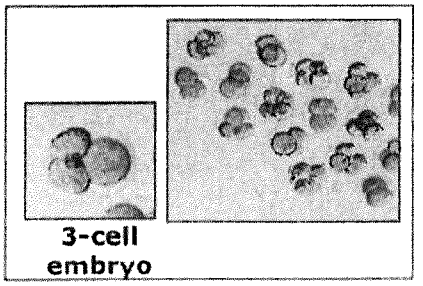


FIG. 5E

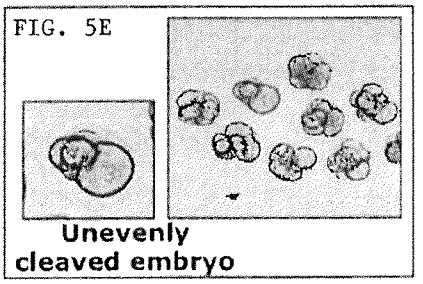
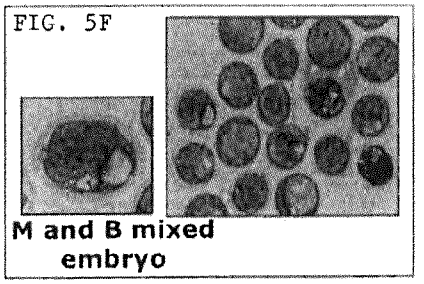


FIG. 5F



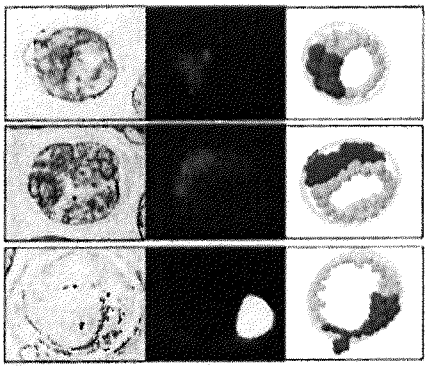
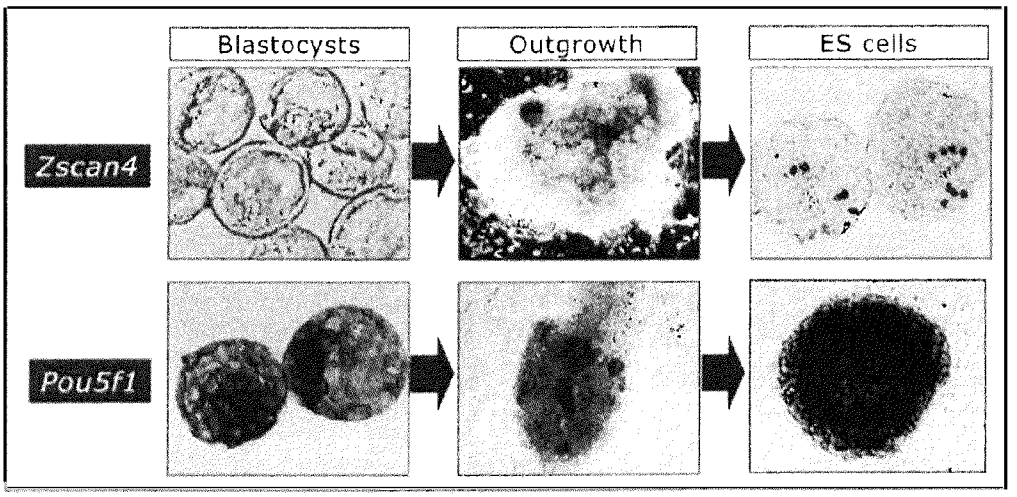


FIG. 5G

FIG. 6A


Blastocysts
Outgrowth
ES cells
Zscan4
Pou5f1

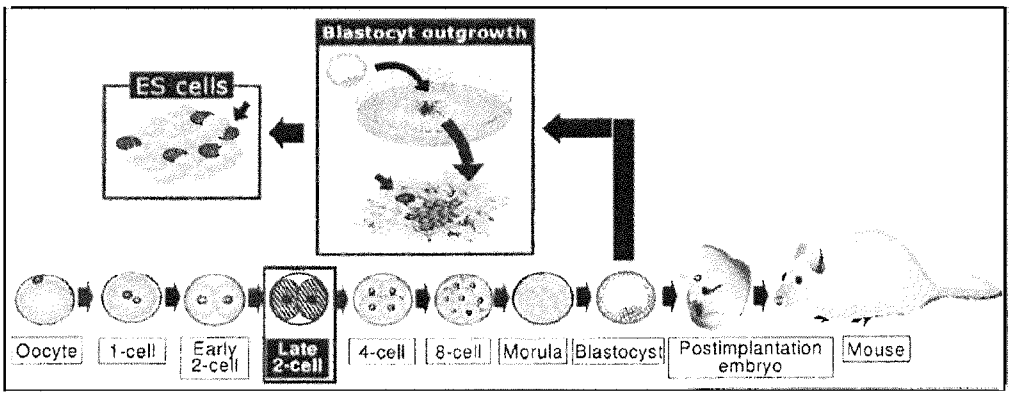

Blastocyt outgrowth
ES cells
Oocyte
1-cell
Early 2-cell
Late 2-cell
4-cell
8-cell
Morula
Blastocyst
Postimplantation embryo
Mouse

FIG. 6B

FIG. 7A

cDNA (length)	Human *ZSCAN4* (2230bp)	Mouse *Zscan4c* (2275bp)	Mouse *Zscan4d* (2268bp)	Mouse *Zscan4f* (2273bp)
ZSCAN4	-	54	55	54
Zscan4c		-	97	99
Zscan4d			-	97
Zscan4f				-

FIG. 7B

CDS (length)	Human *ZSCAN4* (1302bp)	Mouse *Zscan4c* (1518bp)	Mouse *Zscan4d* (1518bp)	Mouse *Zscan4f* (1518bp)
ZSCAN4	-	65	66	65
Zscan4c		-	98	99
Zscan4d			-	98
Zscan4f				-

FIG. 7C

Protein (length)	Human ZSCAN4 (433aa)	Mouse ZSCAN4C (506aa)	Mouse ZSCAN4D (506aa)	Mouse ZSCAN4F (506aa)
ZSCAN4	-	45	44	44
ZSCAN4C		-	95	99
ZSCAN4D			-	94
ZSCAN4F				-

FIG. 7D

SCAN Domain (length)	Human ZSCAN4 (96aa)	Mouse ZSCAN4C (99aa)	Mouse ZSCAN4D (99aa)	Mouse ZSCAN4F (99aa)
ZSCAN4	-	50	50	50
ZSCAN4C		-	98	100
ZSCAN4D			-	98
ZSCAN4F				-

FIG. 7E

ZFP Domain (length)	Human ZSCAN4 (107aa)	Mouse ZSCAN4C (109aa)	Mouse ZSCAN4D (109aa)	Mouse ZSCAN4F (109aa)
ZSCAN4	-	59	58	59
ZSCAN4C		-	99	100
ZSCAN4D			-	99
ZSCAN4F				-

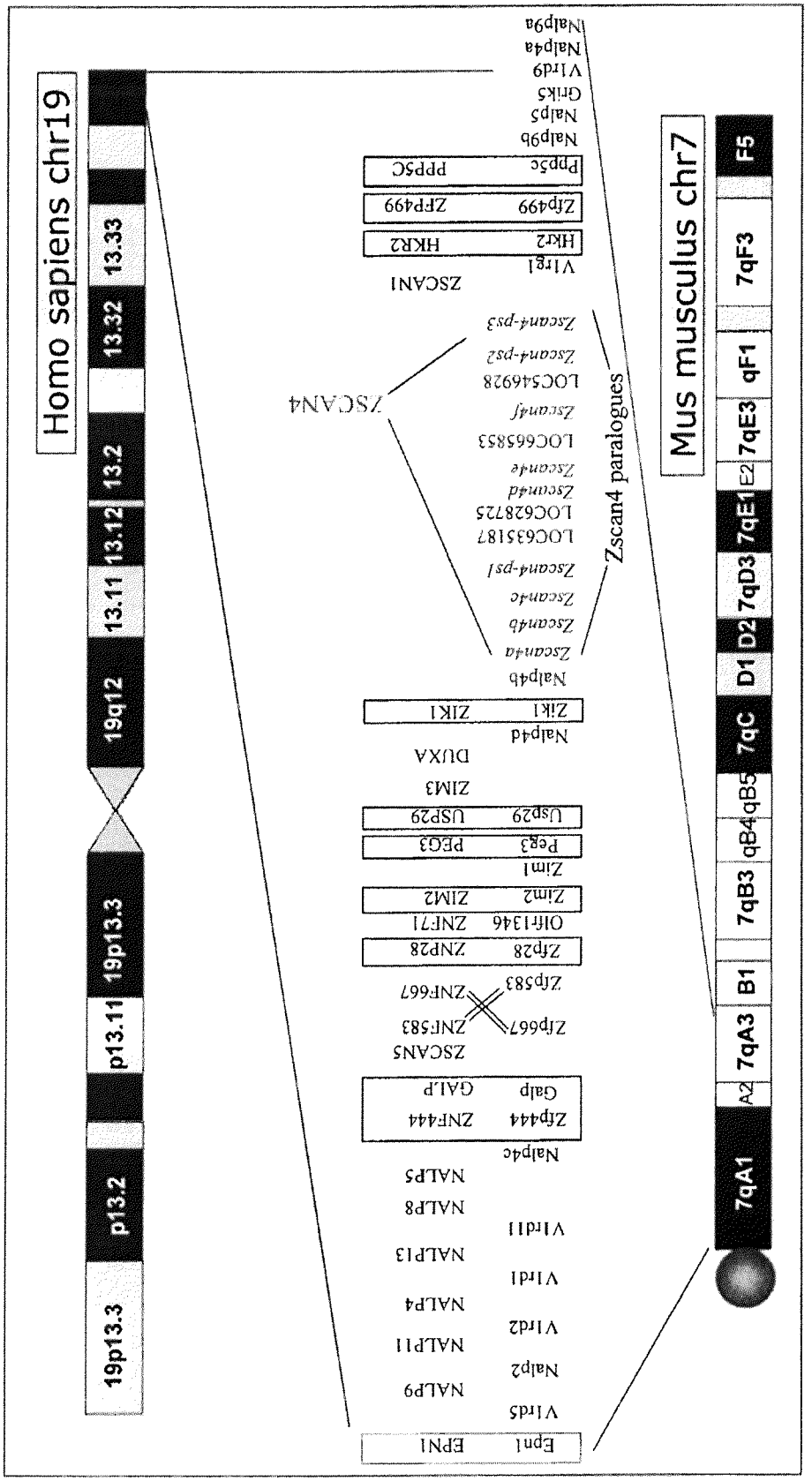

Homo sapiens chr19
19p13.3
19p13.2
19p13.11
19p13.3
19q12
13.11
13.12
13.2
13.32
13.33
Mus musculus chr7
7qA1
7qA3
7qB3
7qC
7qD3
7qE1
7qE3
7qF1
7qF3
F5
Zscan4 paralogues
ZSCAN4
ZSCAN1
Zscan4-ps3
Zscan4-ps2
LOC546928
Zscan4f
LOC665853
Zscan4e
Zscan4d
LOC628725
LOC635187
Zscan4-ps1
Zscan4c
Zscan4b
Zscan4a
Naip4b
Naip4d
Zik1 ZIK1
DUXA
ZIM3
Usp29 USP29
Peg3 PEG3
Zim1
Zim2 ZIM2
Olfr1346 ZNF71
Zfp28 ZNP28
Zfp583 ZNF667
Zfp667 ZNF583
ZSCAN5
Galp GALP
Zfp444 ZNF444
Naip4c
NAIP5
NAIP8
Vlrd11
NAIP13
Vlrd1
NAIP4
Vlrd2
NAIP11
Naip2
NAIP9
Vlrd5
Epn1 EPN1
Naip9a
Naip4a
Vlrd9
Grik5
Naip5
Naip9b
Ppp5c PPP5C
Zfp499 ZPP499
Hkr2 HKR2
Vlrg1

FIG. 8

FIG. 9A

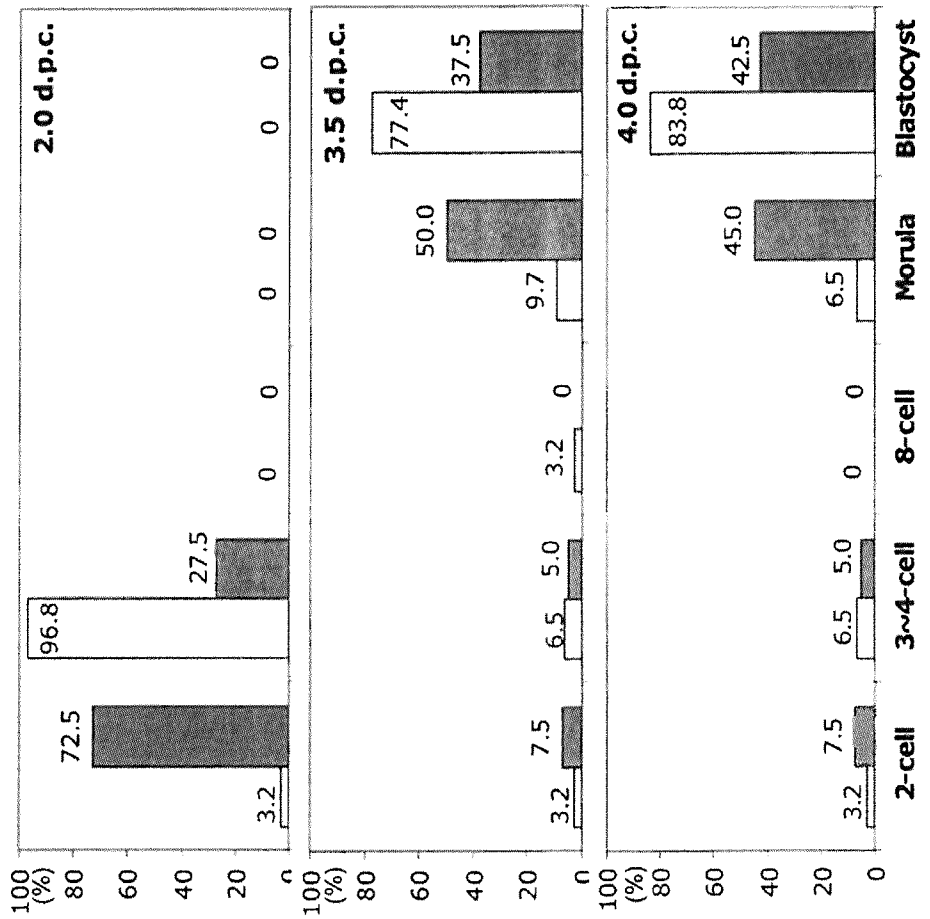

2.0 d.p.c.
3.5 d.p.c.
4.0 d.p.c.
100
80
60
40
20
0
(%)
3.2
72.5
96.8
27.5
0
0
0
0
0
0
3.2
7.5
6.5
5.0
0
3.2
9.7
50.0
77.4
37.5
3.2
7.5
6.5
5.0
0
0
6.5
45.0
83.8
42.5
2-cell
3~4-cell
8-cell
Morula
Blastocyst

FIG. 9B

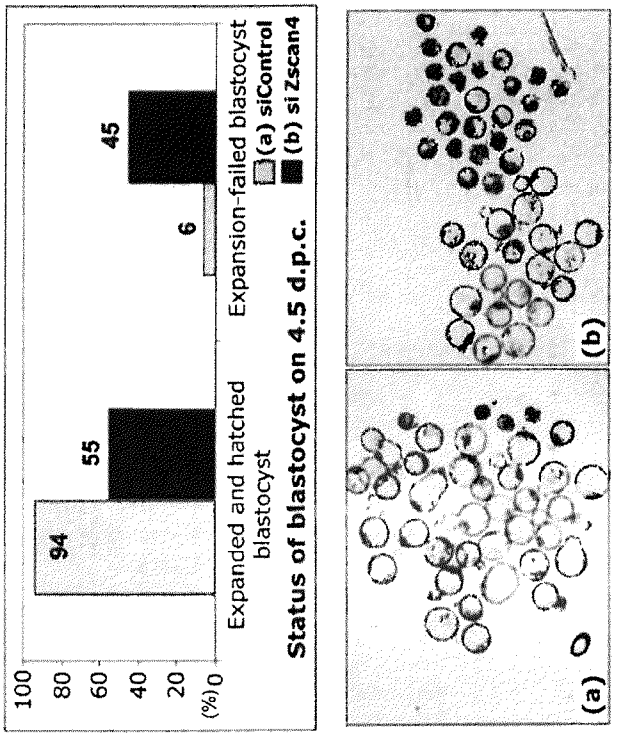

100
80
60
40
20
0
(%)
94
55
6
45
Expanded and hatched blastocyst
Expansion-failed blastocyst
(a) siControl
(b) si Zscan4
Status of blastocyst on 4.5 d.p.c.
(a)
(b)

FIG. 10A

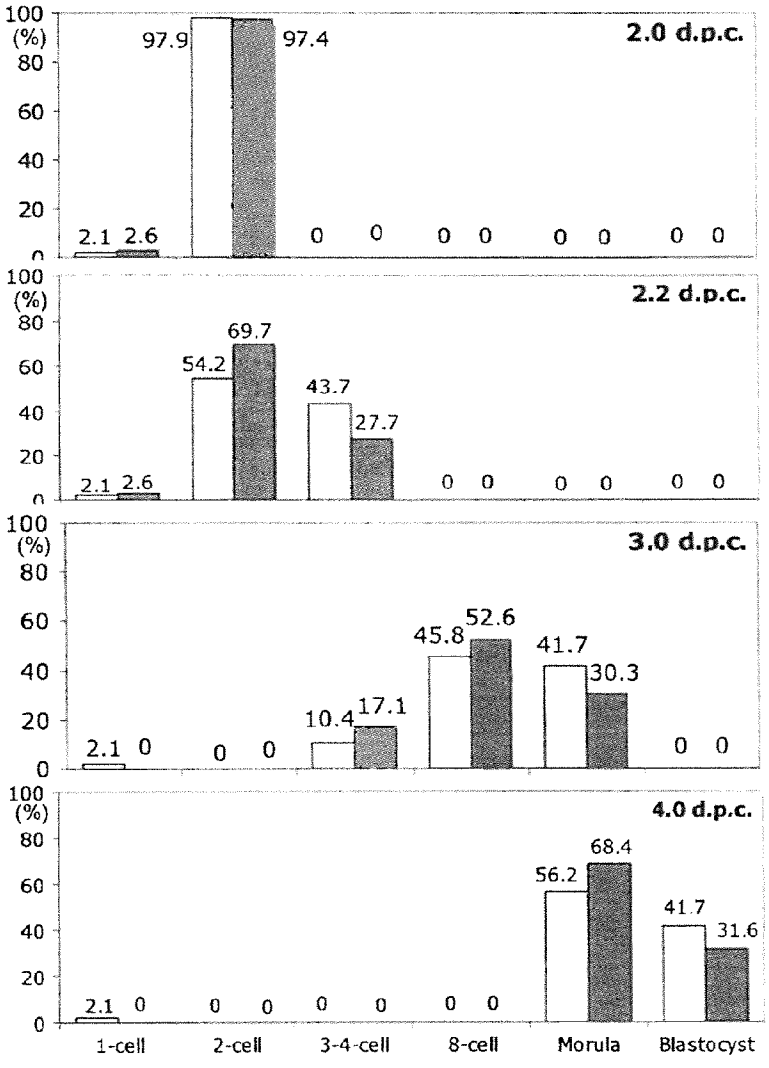
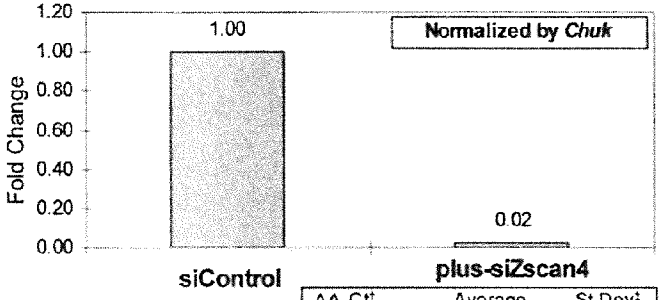
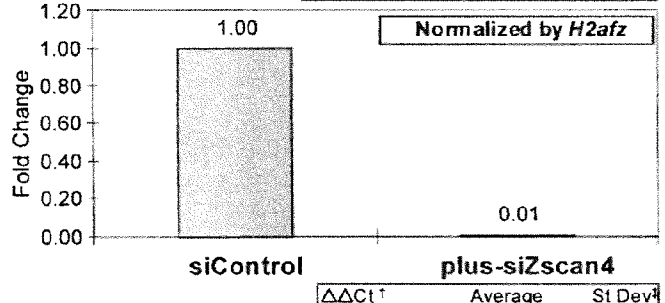


FIG. 10B



ΔΔ Ct[†]	Average	St Dev[‡]
Scramble	1.00	0
plus-siZscan4	0.02	0.02

FIG. 10C



ΔΔCt[†]	Average	St Dev[‡]
Scramble	1.00	0
plus-siZscan4	0.01	0.02

FIG. 10D

		Developmental delayed embryos of					Average of developmental delayed embryos of	
		Scramble		*Zscan4*				
		C1	*C2*	*C1*	*C2*	*C3*	Scramble	*Zscan4*
Zscan4	Ct[†]	24.00	220.5	30.91	27.48	30.61	23.02	29.67
	St Dev[‡]	0.02	0.01	0.34	0.05	0.24	1.38	1.90
Chuk	Ct[†]	30.83	31.67	31.98	31.22	30.73	31.25	31.31
	St Dev[‡]	0.83	0.48	0.30	0.86	0.17	0.59	0.63
H2afz	Ct[†]	20.91	20.89	20.29	20.47	19.04	20.90	19.93
	St Dev[‡]	0.09	0.45	0.09	0.03	0.05	0.02	0.78

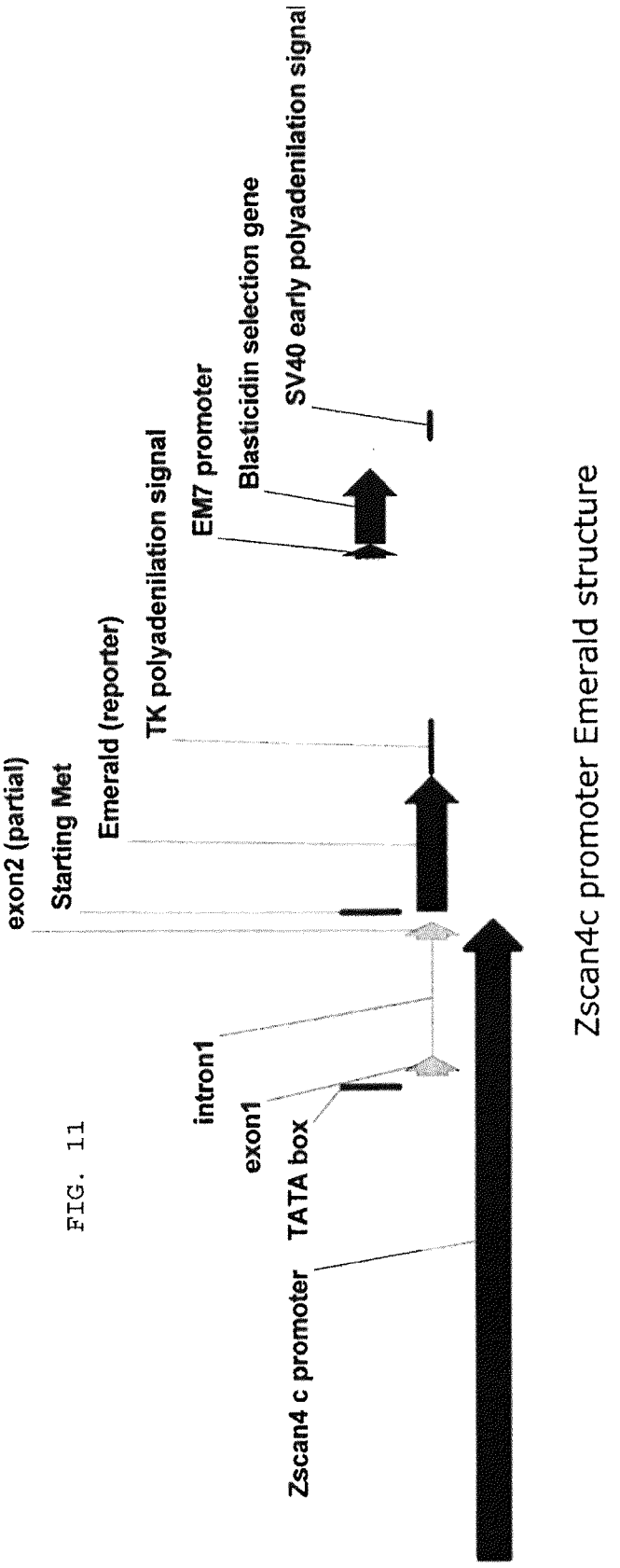

Zscan4c promoter Emerald structure
6017 bp
Zscan4 c promoter
TATA box
exon1
intron1
exon2 (partial)
Starting Met
Emerald (reporter)
TK polyadenilation signal
EM7 promoter
Blasticidin selection gene
SV40 early polyadenilation signal

FIG. 11

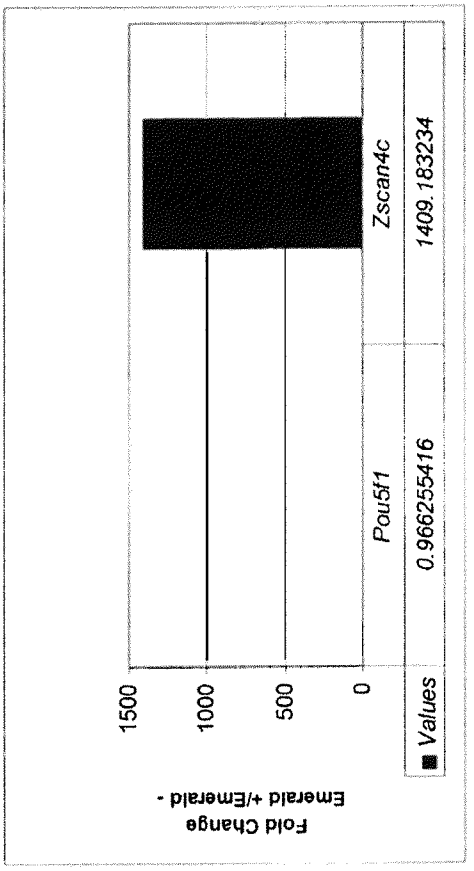


FIG. 12B

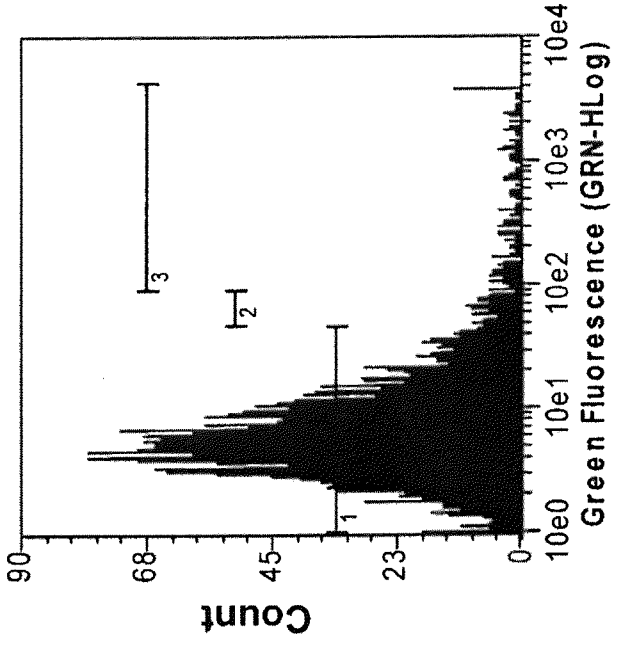


FIG. 12A

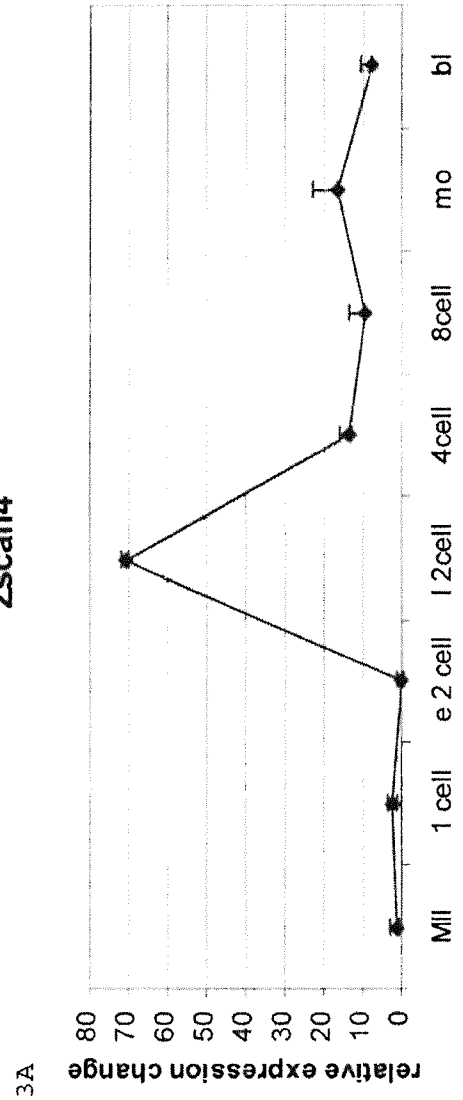


FIG. 13A

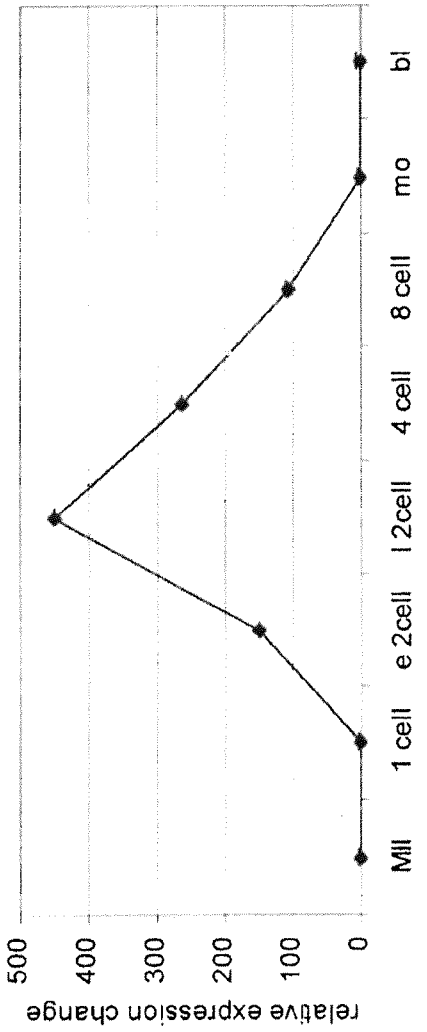


FIG. 13B

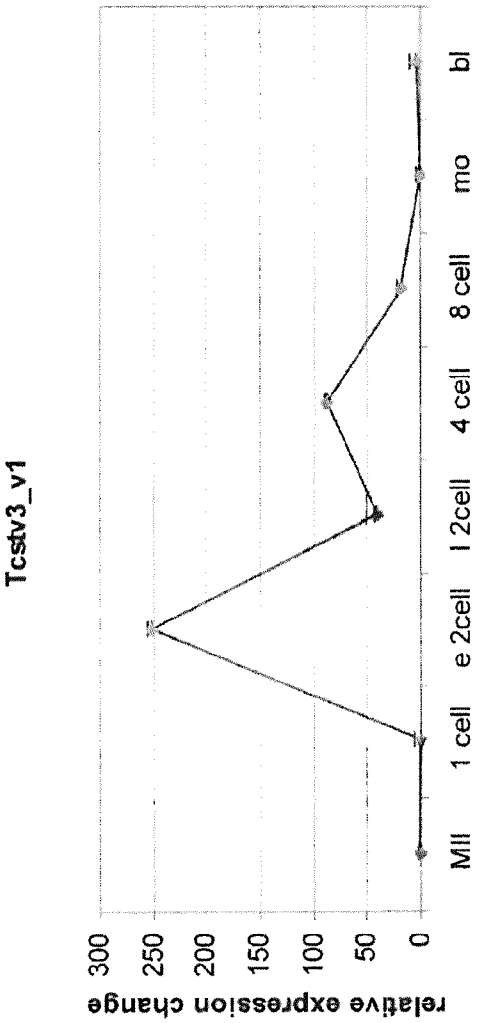


FIG. 13C

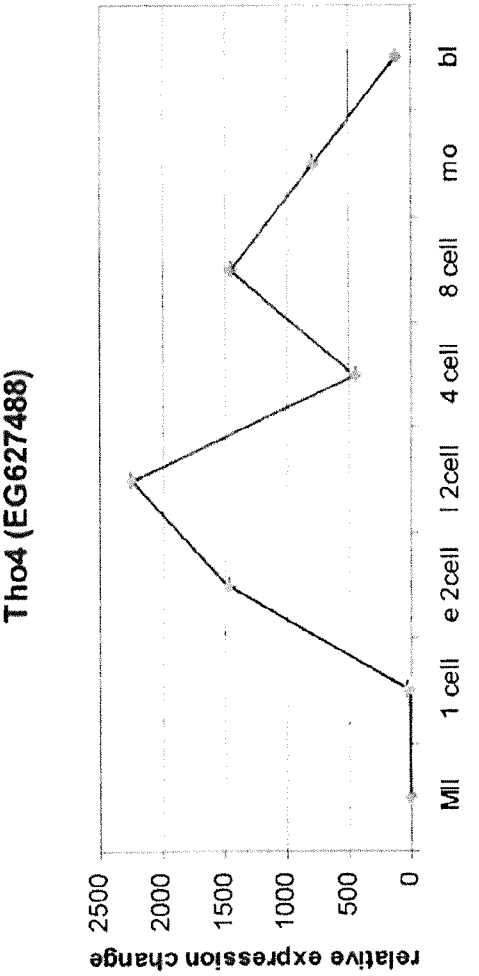


FIG. 13D

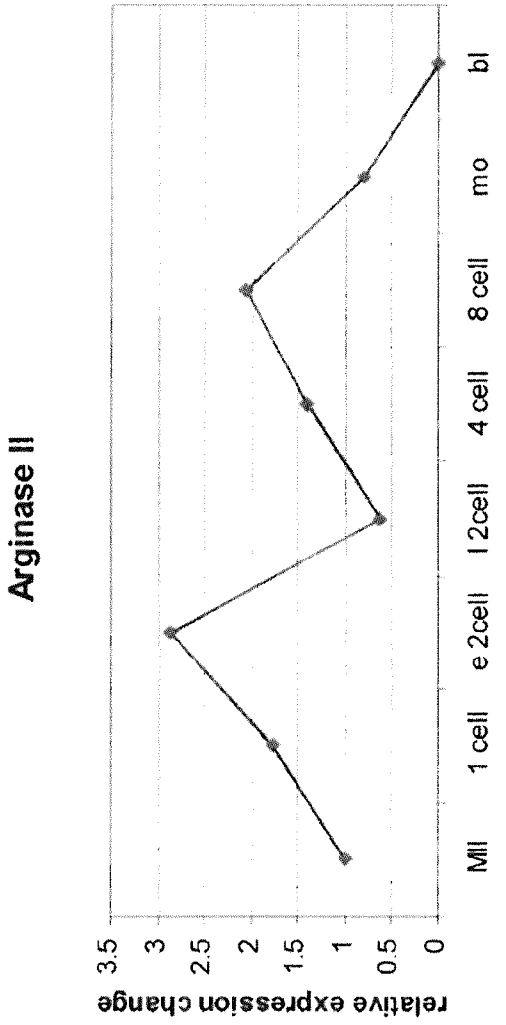


FIG. 13E

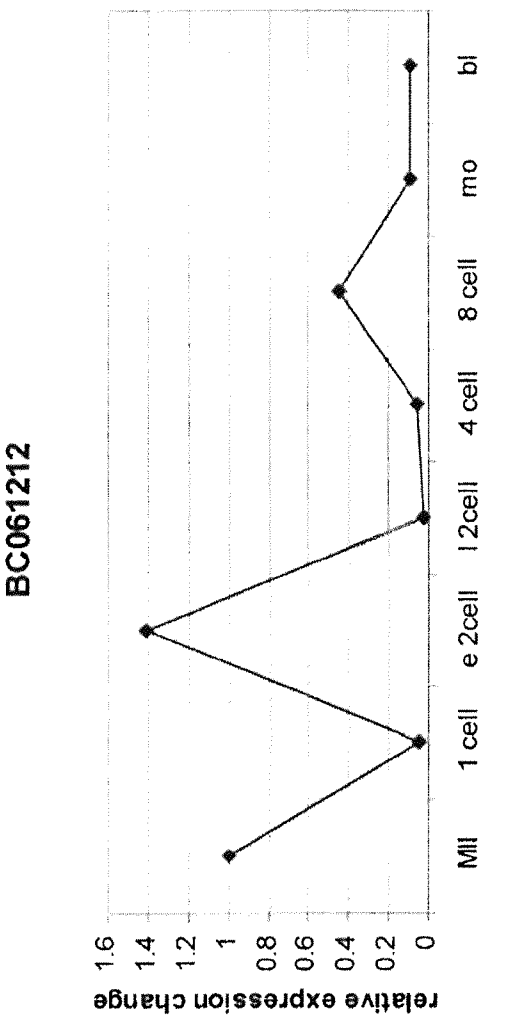


FIG. 13F

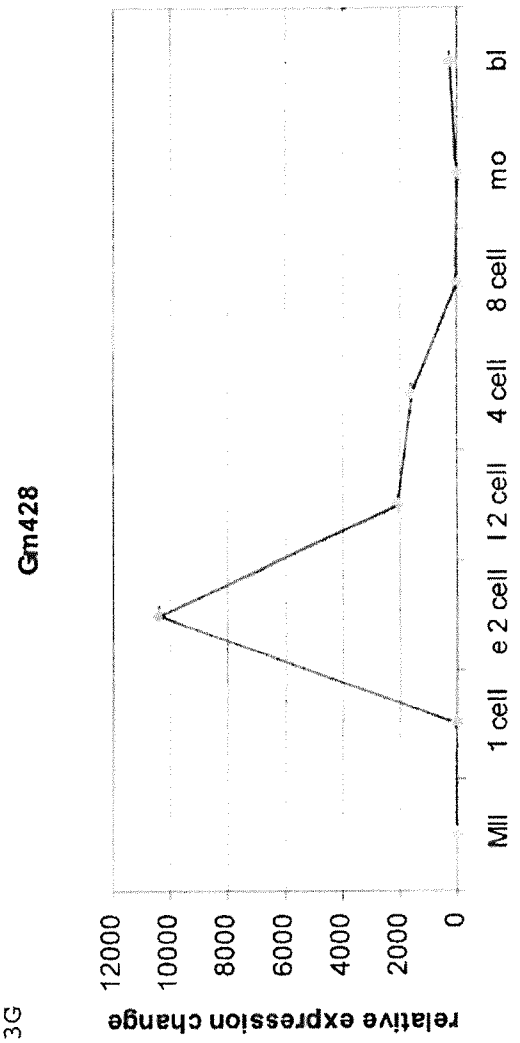

Gm428
relative expression change
12000
10000
8000
6000
4000
2000
0
MII
1 cell
e 2 cell
l 2 cell
4 cell
8 cell
mo
bl

FIG. 13G

METHODS FOR MODULATING EMBRYONIC STEM CELL DIFFERENTIATION

CROSS REFERENCE TO RELATED APPLICATIONS

This is a divisional of U.S. application Ser. No. 12/529,004, filed Aug. 27, 2009, now abandoned, which is the U.S. National Stage of International Application No. PCT/US2008/058261, filed Mar. 26, 2008, published in English under PCT Article 21(2), which claims the benefit of U.S. Provisional Application No. 60/920,215, filed Mar. 26, 2007. All of the above-referenced applications are herein incorporated by reference in their entirety.

FIELD

This application relates to the field of cellular differentiation, specifically to the methods of identifying and using a subpopulation of stem cells, which can be identified by the expression of Zscan4 or one or more Zscan4 co-expressed genes described herein, and the methods of inhibiting differentiation and prolonging viability by altering Zscan4. This application also relates to the identification of Trim43 as a gene highly expressed at the morula stage.

BACKGROUND

Stem cells have been identified in several somatic tissues including the nervous system, bone marrow, epidermis, skeletal muscle, and liver. This 'set-aside' population of cells is believed to be responsible for maintaining homeostasis within individual tissues in adult animals. The number of stem cells and their decision to differentiate must be tightly controlled during embryonic development and in the adult animal to avoid premature aging or tumor formation. Different somatic stem cells share the properties of self-renewal and multi-developmental potential, suggesting the presence of common cellular machinery.

Embryonic stem (ES) cells can proliferate indefinitely in an undifferentiated state. Furthermore, ES cells are pluripotent cells, meaning that they can generate all of the cells present in the body (bone, muscle, brain cells, etc.). ES cells have been isolated from the inner cell mass of the developing murine blastocyst (Evans et al., *Nature* 292:154-156, 1981; Martin et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:7634-7636, 1981; Robertson et al., *Nature* 323:445-448, 1986; Doetschman et al., *Nature* 330:576-578, 1987; and Thomas et al., *Cell* 51:503-512, 1987; U.S. Pat. No. 5,670,372). Additionally, human cells with ES cell properties have recently been isolated from the inner blastocyst cell mass (Thomson et al., *Science* 282:1145-1147, 1998) and developing germ cells (Shamblott et al., *Proc. Natl. Acad. Sci. U.S.A.* 95:13726-13731, 1998) (see also U.S. Pat. No. 6,090,622, PCT Publication Nos. WO 00/70021 and WO 00/27995).

There is growing interest in the analysis of patterns of gene expression in cells, such as stem cells. However, few studies have identified an individual gene product that functions in the complex network of signals in developing tissues to inhibit differentiation and increase proliferation.

SUMMARY

Described herein is the identification of Zscan4 as a gene specifically expressed during the 2-cell embryonic stage and in embryonic stem cells. Further described herein is the identification of Zscan4 co-expressed genes which exhibit a similar expression pattern as Zscan4 in the developing embryo. Also described herein is the identification of Trim43 as a gene abundantly expressed at the morula stage of embryonic development.

Provided herein are methods of inhibiting differentiation of a stem cell comprising increasing the expression of Zscan4 in the stem cell. In one embodiment, inhibiting differentiation of the stem cell increases viability of the stem cells. In another embodiment, inhibiting differentiation of the stem cell prevents senescence of the stem cell. As described herein, the stem cell can be any type of stem cell, including, but not limited to, an embryonic stem cell, an embryonic germ cell, a germline stem cell or a multipotent adult progenitor cell.

Also provided herein is a method of promoting blastocyst outgrowth of an embryonic stem cell, comprising increasing the expression of Zscan4 in the embryonic stem cell, thereby promoting blastocyst outgrowth of the embryonic stem cell.

Further provided is a method of identifying an undifferentiated subpopulation of stem cells expressing Zscan4, comprising transfecting stem cells with an expression vector comprising a Zscan4 promoter and a reporter gene, wherein expression of the reporter gene indicates Zscan4 is expressed in the subpopulation of stem cells. In one embodiment, the promoter is a Zscan4c promoter.

An isolated expression vector comprising a Zscan4 promoter operably linked to a heterologous polypeptide is also provided. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In another embodiment, the heterologous polypeptide is a marker, enzyme or fluorescent protein. Also provided is an expression vector comprising a Trim43 promoter operably linked to a heterologous polypeptide. In some embodiments, the Trim43 promoter comprises at least a portion of the nucleic acid sequence set forth as SEQ ID NO: 31. Isolated embryonic stem cells comprising the expression vectors described herein are also provided.

Also provided is a method of identifying an undifferentiated subpopulation of stem cells, wherein the stem cells express Zscan4, comprising detecting expression of one or more of AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1. Isolated stem cells identified according to this method are also provided.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**A is a series of digital images showing the expression profile of Zscan4 during preimplantation development by whole mount in situ hybridization. Hybridizations were performed simultaneously under the same experimental conditions for all preimplantation developmental stages. Images were taken at 200× magnification using phase contrast. Zscan4 shows a transient and high expression in the late 2-cell embryos. Such a high level of expression was not observed in 3-cell (two examples indicated by red arrows) and 4-cell embryos. FIG. **1**B shows a graph of the expression levels of Zscan4 during preimplantation development quantitated by qRT-PCR analysis. Three sets of 10 pooled embryos were collected from each stage (O, oocyte; 1, 1-cell embryo; E2, early 2-cell embryo; L2, late 2-cell embryo; 4, 4-cell embryo; 8, 8-cell embryo; M, morula; and B, blastocyst) and used for qRT-PCR analysis. The expression levels of Zscan4 were normalized to Chuk control, and the average expression lev-

els at each stage are represented as a fold change compared to the expression level in oocytes.

FIG. **2**A shows diagrams of the exon-intron structures of nine Zscan4 paralogs. New proposed gene symbols are shown in bold italics with the current gene symbols. FIG. **2**B illustrates the putative protein structures of Zscan4 paralogs, and shows predicted domains.

FIG. **3**A is a diagram that illustrates the genomic structure of the Zscan4 locus (encompassing 850 kb on Chromosome 7). The top panel shows genes near the Zscan4 locus. The lower panel shows nine Zscan4 paralogous genes and their characteristic features. Six other genes (LOCs) are predicted in this region, but unrelated to Zscan4. FIG. **3**B is a diagram that depicts the TaqI-, MspI-, or TaqI/MspI-digested DNA fragment sizes predicted from the genome sequences assembled from individual BAC sequences. FIG. **3**C is a digital image that shows the Southern blot analysis of C57BL/6J genomic DNAs digested with TaqI, MspI, or TaqI/MspI restriction enzymes. Sizes of all DNA fragments hybridized with a Zscan4 probe (containing only exon 3 from cDNA clone C0348C03) matched with those predicted in FIG. **3**B, validating the manually assembled sequences.

FIG. **4**A is a table showing the three types of siRNA technologies used for the analysis of Zscan4 in preimplantation embryos and their target sequences (SEQ ID NOs: 54-59). FIG. **4**B is a diagram that illustrates the locations of siRNA target sequences in the Zscan4 cDNA. FIG. **4**C is a series of digital images showing the development of shZscan4-injected embryos. The morphology of representative embryos is shown. Stages of shZscan4-injected and shControl-injected embryos were assessed at 61 hrs, 80 hrs, 98 hrs and 108 hrs post-hCG injections. FIG. **4**D is a series of graphs showing the percentage of shZscan4- and shControl-injected embryos at each developmental stage. shZscan4-injected (grey bars) and shControl-injected (white bars) were staged and counted at 61 hrs, 80 hrs, 98 hrs and 108 hrs post-hCG injections (M=morula; B=blastocyst). FIG. **4**E is a graph showing the transcript levels of Zscan4 in shControl-injected and shZscan4-injected 2-cell embryos by qRT-PCR analysis. The expression levels were normalized by Eef1a1.

FIGS. **5**A-**5**C are a series of graphs indicating the number of embryos at each developmental stage following injection with shZscan4. Embryos received shZscan4-injection in the nucleus of one blastomere of early 2-cell embryos. The stages of shZscan4-(gray) and shControl-(white) microinjected embryos were assessed at 52 hrs, 74 hrs and 96 hrs post-hCG injections. FIGS. **5**D-**5**F show photographs of a 3-cell embryo (D), an unevenly cleaved embryo (E) and a mixed morula and blastocyst like embryo (F). The 3-cell embryo has one blastomere that remained at the size of a 2-cell stage blastomere and two smaller blastomeres with the size of 4-cell stage blastomeres. The 5-cell embryo has one delayed blastomere and four smaller blastomeres with the size of 8-cell blastomeres. These embryos eventually formed blastocyst-like structures, but seemed to be a mixture of a blastocyst-like cell mass and a morula-like cell mass. The morula-like cell mass was developed from one blastomere receiving shZscan4 injection, as shown by the presence of GFP, which was carried in the shZscan4 plasmid (FIG. **5**G). Magnification is 200×.

FIG. **6**A is an image that illustrates the expression of Zscan4 and Pou5f1 in blastocysts, blastocyst outgrowth and ES cells by whole mount in situ hybridization. FIG. **6**B is a schematic illustration of the Zscan4 expression patterns.

FIGS. **7**A-**7**E is a series of tables comparing nucleotide and amino acid sequence similarity (percent identity) among human ZSCAN4, mouse Zscan4c, Zscan4d, and Zscan4f genes.

FIG. **8** is an illustration showing the Zscan4 syntenic regions of mouse and human genomes.

FIGS. **9**A-**9**B is a series of graphs and photographs showing the development of embryos that received a siZscan4-injection in the cytoplasm. FIG. **9**A shows the percentage of embryos at each developmental stage for siControl-injected embryos (white bar) and siZscan4-injected embryos (gray bar) at 2.0, 3.5 and 4.0 d.p.c. FIG. **9**B shows the percentage of expanded and hatched blastocysts at 4.5 d.p.c. in siControl-injected embryos (gray bar; photograph (a)) and siZscan4-injected embryos (black bar; photograph (b)).

FIGS. **10**A-**10**D are a series of graphs and a table showing the development of embryos that received plus-siZscan4-injection in cytoplasm. FIG. **10**A shows the percentage of embryos at each developmental stage for siControl-injected embryos (white bar) and plus-siZscan4-injected embryos (gray bar) at 2.0, 2.2, 3.0, and 4.0 days post coitus. FIGS. **10**B and **10**C show the transcript levels of Zscan4 in siControl-injected embryos and plus-siZscan4-injected embryos, measured by qRT-PCR analysis and normalized by Chuk (FIG. **10**B) and H2afz (FIG. **10**C). FIG. **10**D provides the raw data of 3 biological replications of qRT-PCR analysis. †, the mean value of the cycle threshold for each biological replicate; ‡, the standard deviation.

FIG. **11** is an illustration depicting the expression vector comprising the Zscan4c promoter sequence and reporter gene Emerald. The sequence of the expression vector is set forth as SEQ ID NO: 28.

FIG. **12**A is a fluorescence activated cell sorting (FACS) graph showing a subpopulation of mouse ES expressing Zscan4. Mouse ES cells were transfected with an expression vector comprising a Zscan4c promoter and a fluorescent reporter gene (Emerald). Expression of the reporter gene in a cell (an Emerald-positive cell) indicates the cell expresses Zscan4. FIG. **12**B is a graph showing expression levels of Zscan4c and Pou5f1 in the subpopulation of ES cells identified as Emerald-positive. The Y-axis represents the fold difference in gene expression between Emerald-positive and Emerald-negative cells.

FIGS. **13**A-G are graphs showing expression profiles of Zscan4 and six genes co-expressed with Zscan4 in a subpopulation of ES cells. Shown are the expression profiles of Zscan4 (A), AF067063 (B), Tcstv3 (C), Tho4 (D), Arginase II (E), BC061212 (F) and Gm428 (G)) in metaphase II oocytes (MII), 1 cell embryos, early 2 cell (e 2 cell) embryos, late 2 cell (l 2 cell) embryos, 4 cell embryos, 8 cell embryos, morula (mo) and blastocyts (bl).

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file, created on Dec. 14, 2011, 170 KB, which is incorporated by reference herein. In the accompanying sequence listing:

SEQ ID NOs: 1 and 2 are the nucleotide sequences of forward and reverse PCR primers for amplification of Zscan4d from 2-cell embryos.

SEQ ID NOs: 3 and 4 are the nucleotide sequences of PCR primers for amplifying a probe designed to contain exon 3 of Zscan4.

SEQ ID NO: 5 is the nucleotide sequence of the Zscan4 PCR and sequencing primer Zscan4_For.

SEQ ID NO: 6 is the nucleotide sequence of the Zscan4 PCR and sequencing primer Zscan4_Rev.

SEQ ID NO: 7 is the nucleotide sequence of the Zscan4 sequencing primer Zscan4_400Rev.

SEQ ID NO: 8 is the nucleotide sequence of the Zscan4 sequencing primer Zscan4_300Rev.

SEQ ID NO: 9 is the nucleotide sequence of the shZscan4 siRNA. SEQ ID NO: 10 is the nucleotide sequence of the siControl siRNA.

SEQ ID NO: 11 is the nucleotide sequence of Genbank Accession No. BC050218 (deposited Apr. 3, 2003), a cDNA clone derived from ES cells (Clone No. C0348C03).

SEQ ID NO: 12 is the nucleotide sequence of Zscan4-ps1.

SEQ ID NO: 13 is the nucleotide sequence of Zscan4-ps2.

SEQ ID NO: 14 is the nucleotide sequence of Zscan4-ps3.

SEQ ID NOs: 15 and 16 are the nucleotide and amino acid sequences of Zscan4a.

SEQ ID NOs: 17 and 18 are the nucleotide and amino acid sequences of Zscan4b.

SEQ ID NOs: 19 and 20 are the nucleotide and amino acid sequences of Zscan4c.

SEQ ID NOs: 21 and 22 are the nucleotide and amino acid sequences of Zscan4d.

SEQ ID NOs: 23 and 24 are the nucleotide and amino acid sequences of Zscan4e.

SEQ ID NOs: 25 and 26 are the nucleotide and amino acid sequences of Zscan4f.

SEQ ID NO: 27 is the nucleotide sequence of Genbank Accession No. XM_145358, deposited Jan. 10, 2006, incorporated by reference herein.

SEQ ID NO: 28 is the nucleotide sequence of the Zscan4-Emerald expression vector.

SEQ ID NOs: 29 and 30 are the nucleotide and amino acid sequences of human ZSCAN4 (Genbank Accession No. NM_152677, deposited Sep. 6, 2002, incorporated by reference herein).

SEQ ID NO: 31 is the nucleotide sequence of the Trim43 promoter.

SEQ ID NOs: 32 and 33 are the nucleotide and amino acid sequences of Trim43.

SEQ ID NOs: 34 and 35 are the nucleotide and amino acid sequences of AF067063, Genbank Accession No. NM_001001449, deposited May 29, 2004, incorporated by reference herein.

SEQ ID NOs: 36 and 37 are the nucleotide and amino acid sequences of BC061212, Genbank Accession No. NM_198667.1, deposited Nov. 15, 2003, incorporated by reference herein.

SEQ ID NOs: 38 and 39 are the nucleotide and amino acid sequences of Gm428, Genbank Accession No. NM_001081644, deposited Feb. 22, 2007, incorporated by reference herein.

SEQ ID NOs: 40 and 41 are the nucleotide and amino acid sequences of Arginase II, Genbank Accession No. NM_009705, deposited Jan. 26, 2000, incorporated by reference herein.

SEQ ID NOs: 42 and 43 are the nucleotide and amino acid sequences of Tcstv1, Genbank Accession No. NM_018756, deposited Jul. 12, 2007, incorporated by reference herein.

SEQ ID NOs: 44 and 45 are the nucleotide and amino acid sequences of Tcstv3, Genbank Accession No. NM_153523, deposited Oct. 13, 2002, incorporated by reference herein.

SEQ ID NOs: 46 and 47 are the nucleotide and amino acid sequences of Tho4, Genbank Accession No. XM_902103, deposited Dec. 2, 2005, incorporated by reference herein.

SEQ ID NOs: 48 and 49 are the nucleotide and amino acid sequences of Eif1a, Genbank Accession No. NM_010120, deposited Aug. 3, 2002, incorporated by reference herein.

SEQ ID NOs: 50 and 51 are the nucleotide and amino acid sequences of EG668777, Genbank Accession No. XM_001003556, deposited Apr. 27, 2006, incorporated by reference herein.

SEQ ID NOs: 52 and 53 are the nucleotide and amino acid sequences of Pif1, Genbank Accession No. NM_172453, deposited Dec. 24, 2002, incorporated by reference herein.

SEQ ID NO: 54 is the nucleotide sequence of the Plus-siZscan4 (J-064700-05) target sequence.

SEQ ID NO: 55 is the nucleotide sequence of the Plus-siZscan4 (J-064700-06) target sequence.

SEQ ID NO: 56 is the nucleotide sequence of the Plus-siZscan4 (J-064700-07) target sequence.

SEQ ID NO: 57 is the nucleotide sequence of the Plus-siZscan4 (J-064700-08) target sequence.

SEQ ID NO: 58: is the nucleotide sequence of the siZscan4 target sequence.

SEQ ID NO: 59 is the nucleotide sequence of the of shZscan4 target sequence.

SEQ ID NO: 60 is the nucleotide consensus sequence of nucleotides 1-1848 of Zscan4c, Zscan4d and Zscan4f.

DETAILED DESCRIPTION

I. Abbreviations

CDS	Coding sequence
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
d.p.c.	Days post coitus
EC	Embryonic carcinoma
EG	Embryonic germ
ES	Embryonic stem
GS	Germline stem
GFP	Green fluorescent protein
hCG	Human chorionic gonadotropin
ICM	Inner cell mass
IVF	In vitro fertilization
LIF	Leukemia inhibitory factor
maGSC	Multipotent adult germline stem cell
MAPC	Multipotent adult progenitor cell
PCR	Polymerase chain reaction
qRT-PCR	Quantitative reverse-transcriptase polymerase chain reaction
RNA	Ribonucleic acid
siRNA	small interfering RNA
TS	Trophoblast stem
USSC	Unrestricted somatic stem cell
ZGA	Zygotic genome activation

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al. (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the invention, the following explanations of specific terms are provided:

Alter: A change in an effective amount of a substance of interest, such as a polynucleotide or polypeptide. The amount of the substance can be changed by a difference in the amount of the substance produced, by a difference in the amount of the substance that has a desired function, or by a difference in the activation of the substance. The change can be an increase or a decrease. The alteration can be in vivo or in vitro. In several embodiments, altering an effective amount of a polypeptide or polynucleotide is at least about a 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% increase or decrease in the effective amount (level) of a substance. Altering an effective amount of a polypeptide or polypeptide includes increasing the expression of Zscan4 in a cell. In another embodiment, an alteration in a polypeptide or polynucleotide affects a physiological property of a cell, such as the differentiation, proliferation, or viability of the cell. For example, increasing expression of Zscan4 in a stem cell inhibits differentiation and promotes viability of the stem cell.

Blastocyst: The structure formed in early mammalian embryogenesis, after the formation of the blastocele, but before implantation. It possesses an inner cell mass, or embryoblast, and an outer cell mass, or trophoblast. The human blastocyst comprises 70-100 cells. As used herein, blastocyst outgrowth refers to the process of culturing embryonic stem cells derived from the inner cell mass of a blastocyst. Promoting blastocyst outgrowth refers to enhancing the viability and proliferation of embryonic stem cells derived from the blastocyst.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences that determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Co-expressed: In the context of the present disclosure, genes that are "co-expressed" with Zscan4 (also referred to as "Zscan4 co-expressed genes") are genes that exhibit a similar expression pattern as Zscan4 during embryonic development and in ES cells. Specifically, the co-expressed genes are expressed in the same undifferentiated subpopulation of ES cells as Zscan 4, and during embryonic development, these genes are most abundantly expressed at the 2-cell stage. Nine co-expressed genes are described herein, including AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1. However, co-expressed genes are not limited to those disclosed herein, but include any genes exhibiting an expression pattern similar to Zscan4.

AF067063 encodes hypothetical protein LOC380878. The full length cDNA sequence of AF067063 (SEQ ID NO: 34) is 886 base pairs in length and is organized into three exons encoding several hypothetical proteins (for example, SEQ ID NO: 35), which appear to be mouse specific.

BC061212 encodes a protein belonging to the PRAME (preferentially expressed antigen melanoma) family. The full length cDNA sequence of BC061212 (SEQ ID NO: 36) is 1625 base pairs in length and is organized into four exons, encoding a protein of 481 residues in length (SEQ ID NO: 37).

Gm428 (gene model 428) encodes a hypothetical protein. The full length cDNA sequence of Gm428 (SEQ ID NO: 38) is 1325 base pairs in length and is organized into five exons encoding a protein of 360 residues in length (SEQ ID NO: 39).

Arginase II belongs to the Arginase family and may play a role in the regulation of extra-urea cycle arginine metabolism, and in down-regulation of nitric oxide synthesis. The full length cDNA sequence of Arginase II (SEQ ID NO: 40) is 1415 base pairs in length and is organized into eight exons encoding a protein of 354 residues in length (SEQ ID NO: 41).

Tsctv1 and Tsctv3 are splice variants. The full length cDNA of Tsctv1 (SEQ ID NO: 42) is 858 base pairs in length and contains two exons encoding a protein of 171 residues (SEQ ID NO: 43). The full length cDNA sequence of Tsctv3 (SEQ ID NO: 44) is 876 base pairs in length and contains one exon encoding a protein of 169 residues (SEQ ID NO: 45). This family of proteins consists of several hypothetical proteins of approximately 170 residues in length and appears to be mouse-specific.

Tho4 (also called EG627488) encodes a protein with an RNA recognition motif (RRM) involved in regulation of alternative splicing, and protein components of small nuclear ribonucleoproteins (snRNPs). The full length cDNA sequence of Tho4 (SEQ ID NO: 46) is 811 base pairs in length and is organized into three exons encoding a protein of 163 residues in length (SEQ ID NO: 47).

Eif1a belongs to the eukaryotic translation initiation factor family. The full length cDNA sequence of Eif1a (SEQ ID NO: 48) is 2881 base pairs in length and encodes a protein of 144 amino acids (SEQ ID NO: 49).

EG668777 is a predicted gene having similarity to retinoblastoma-binding protein 6, isoform 2. The full length cDNA sequence of EG668777 is 1918 base pairs in length (SEQ ID NO: 50) and contains one exon encoding a protein of 547 residues (SEQ ID NO: 51).

Pif1 is an ATP-dependent DNA helicase. The full length cDNA sequence of Pif1 (SEQ ID NO: 52) is 3680 base pairs in length and contains 12 exons encoding a protein of 650 amino acids (SEQ ID NO: 53).

Degenerate variant: A polynucleotide encoding a polypeptide, such as a Zscan4 polypeptide, that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence of the polypeptide encoded by the nucleotide sequence is unchanged.

Differentiation: Refers to the process by which a cell develops into a specific type of cell (for example, muscle cell, skin cell etc.). In the context of the present disclosure, differentiation of embryonic stem cells refers to the development of the cells toward a specific cell lineage. As a cell becomes more differentiated, the cell loses potency, or the ability to become multiple different cell types. As used herein, inhibiting differentiation means preventing or slowing the development of a cell into a specific lineage.

Embryonic stem (ES) cells: Pluripotent cells isolated from the inner cell mass of the developing blastocyst. "ES cells" can be derived from any organism. ES cells can be derived from mammals. In one embodiment, ES cells are produced from mice, rats, rabbits, guinea pigs, goats, pigs, cows, monkeys and humans. Human and murine derived ES cells are preferred. ES cells are pluripotent cells, meaning that they can generate all of the cells present in the body (bone, muscle, brain cells, etc.). Methods for producing murine ES cells can be found in U.S. Pat. No. 5,670,372, herein incorporated by reference. Methods for producing human ES cells can be found in U.S. Pat. No. 6,090,622, PCT Publication No. WO 00/70021 and PCT Publication No. WO 00/27995, herein incorporated by reference.

Expand: A process by which the number or amount of cells in a cell culture is increased due to cell division. Similarly, the terms "expansion" or "expanded" refers to this process. The terms "proliferate," "proliferation" or "proliferated" may be

used interchangeably with the words "expand," "expansion", or "expanded." Typically, during expansion, the cells do not differentiate to form mature cells.

Expression vector: A vector is a nucleic acid molecule allowing insertion of foreign nucleic acid without disrupting the ability of the vector to replicate and/or integrate in a host cell. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements. An expression vector is a vector that contains the necessary regulatory sequences to allow transcription and translation of inserted gene or genes.

Heterologous: A heterologous polypeptide or polynucleotide refers to a polypeptide or polynucleotide derived from a different source or species.

Host cells: Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Isolated: An isolated nucleic acid has been substantially separated or purified away from other nucleic acid sequences and from the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids. Similarly, "isolated" proteins have been substantially separated or purified from other proteins of the cells of an organism in which the protein naturally occurs, and encompasses proteins prepared by recombination expression in a host cell as well as chemically synthesized proteins.

Multipotent cell: Refers to a cell that can form multiple cell lineages, but not all cell lineages.

Non-human animal: Includes all animals other than humans. A non-human animal includes, but is not limited to, a non-human primate, a farm animal such as swine, cattle, and poultry, a sport animal or pet such as dogs, cats, horses, hamsters, rodents, such as mice, or a zoo animal such as lions, tigers or bears. In one example, the non-human animal is a transgenic animal, such as a transgenic mouse, cow, sheep, or goat. In one specific, non-limiting example, the transgenic non-human animal is a mouse.

Operably linked: A first nucleic acid sequence is operably linked to a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked nucleic acid sequences are contiguous and where necessary to join two protein coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, Pa., 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the fusion proteins herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example, sodium acetate or sorbitan monolaurate.

Pharmaceutical agent: A chemical compound, small molecule, or other composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject or a cell. "Incubating" includes a sufficient amount of time for a drug to interact with a cell. "Contacting" includes incubating a drug in solid or in liquid form with a cell.

Pluripotent cell: Refers to a cell that can form all of an organism's cell lineages (endoderm, mesoderm and ectoderm), including germ cells, but cannot form an entire organisms autonomously.

Polynucleotide: A nucleic acid sequence (such as a linear sequence) of any length. Therefore, a polynucleotide includes oligonucleotides, and also gene sequences found in chromosomes. An "oligonucleotide" is a plurality of joined nucleotides joined by native phosphodiester bonds. An oligonucleotide is a polynucleotide of between 6 and 300 nucleotides in length. An oligonucleotide analog refers to moieties that function similarly to oligonucleotides but have non-naturally occurring portions. For example, oligonucleotide analogs can contain non-naturally occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a phosphorothioate oligodeoxynucleotide. Functional analogs of naturally occurring polynucleotides can bind to RNA or DNA, and include peptide nucleic acid (PNA) molecules.

Polypeptide: A polymer in which the monomers are amino acid residues which are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those which are recombinantly or synthetically produced.

The term "polypeptide fragment" refers to a portion of a polypeptide which exhibits at least one useful epitope. The term "functional fragments of a polypeptide" refers to all fragments of a polypeptide that retain an activity of the polypeptide, such as a Zscan4. Biologically functional fragments, for example, can vary in size from a polypeptide fragment as small as an epitope capable of binding an antibody molecule to a large polypeptide capable of participating in the characteristic induction or programming of phenotypic changes within a cell, including affecting cell proliferation or differentiation. An "epitope" is a region of a polypeptide capable of binding an immunoglobulin generated in response to contact with an antigen. Thus, smaller peptides containing the biological activity of Zscan4, or conservative variants of Zscan4, are thus included as being of use.

The term "soluble" refers to a form of a polypeptide that is not inserted into a cell membrane.

The term "substantially purified polypeptide" as used herein refers to a polypeptide which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. In one embodiment, the polypeptide is at least 50%, for example at least 80% free of other proteins, lipids, carbohydrates or other materials with

which it is naturally associated. In another embodiment, the polypeptide is at least 90% free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. In yet another embodiment, the polypeptide is at least 95% free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated.

Conservative substitutions replace one amino acid with another amino acid that is similar in size, hydrophobicity, etc. Examples of conservative substitutions are shown below:

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Variations in the cDNA sequence that result in amino acid changes, whether conservative or not, should be minimized in order to preserve the functional and immunologic identity of the encoded protein. Thus, in several non-limiting examples, a Zscan4 polypeptide, or other polypeptides disclosed herein, includes at most two, at most five, at most ten, at most twenty, or at most fifty conservative substitutions. The immunologic identity of the protein may be assessed by determining whether it is recognized by an antibody; a variant that is recognized by such an antibody is immunologically conserved. Any cDNA sequence variant will preferably introduce no more than twenty, and preferably fewer than ten amino acid substitutions into the encoded polypeptide. Variant amino acid sequences may be, for example, at least 80%, 90% or even 95% or 98% identical to the native amino acid sequence.

Primers: Short nucleic acids, for example DNA oligonucleotides ten nucleotides or more in length, which are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art.

Probes and primers as used herein may, for example, include at least 10 nucleotides of the nucleic acid sequences that are shown to encode specific proteins. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise 15, 20, 30, 40, 50, 60, 70, 80, 90 or 100 consecutive nucleotides of the disclosed nucleic acid sequences. Methods for preparing and using probes and primers are described in the references, for example Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, N.Y.; Ausubel et al. (1987) *Current Protocols in Molecular Biology*, Greene Publ. Assoc. & Wiley-Intersciences; Innis et al. (1990) *PCR Protocols, A Guide to Methods and Applications*, Innis et al. (Eds.), Academic Press, San Diego, Calif. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.).

When referring to a probe or primer, the term "specific for (a target sequence)" indicates that the probe or primer hybridizes under stringent conditions substantially only to the target sequence in a given sample comprising the target sequence.

Prolonging viability: As used herein, "prolonging viability" of a stem cell refers to extending the duration of time a stem cell is capable of normal growth and/or survival.

Promoter: A promoter is an array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor elements. A "constitutive promoter" is a promoter that is continuously active and is not subject to regulation by external signals or molecules. In contrast, the activity of an "inducible promoter" is regulated by an external signal or molecule (for example, a transcription factor).

Reporter gene: A reporter gene is a gene operably linked to another gene or nucleic acid sequence of interest (such as a promoter sequence). Reporter genes are used to determine whether the gene or nucleic acid of interest is expressed in a cell or has been activated in a cell. Reporter genes typically have easily identifiable characteristics, such as fluorescence, or easily assayed products, such as an enzyme. Reporter genes can also confer antibiotic resistance to a host cell. In one embodiment, the reporter gene encodes the fluorescent protein Emerald. In another embodiment, the reporter gene encodes the fluorescent protein Strawberry.

Senescence: The inability of a cell to divide further. A senescent cell is still viable, but does not divide.

Stem cell: A cell having the unique capacity to produce unaltered daughter cells (self-renewal; cell division produces at least one daughter cell that is identical to the parent cell) and to give rise to specialized cell types (potency). Stem cells include, but are not limited to, ES cells, EG cells, GS cells, MAPCs, maGSCs and USSCs. In one embodiment, stem cells can generate a fully differentiated functional cell of more than one given cell type. The role of stem cells in vivo is to replace cells that are destroyed during the normal life of an animal. Generally, stem cells can divide without limit. After division, the stem cell may remain as a stem cell, become a precursor cell, or proceed to terminal differentiation. A precursor cell is a cell that can generate a fully differentiated functional cell of at least one given cell type. Generally, precursor cells can divide. After division, a precursor cell can remain a precursor cell, or may proceed to terminal differentiation.

Subpopulation: An identifiable portion of a population. As used herein, a "subpopulation" of stem cells expressing Zscan4 is the portion of stem cells in a given population that has been identified as expressing Zscan4. In one embodiment, the subpopulation is identified using an expression vector comprising a Zscan4 promoter and a reporter gene, wherein detection of expression of the reporter gene in a cell indicates the cell expresses Zscan4 and is part of the subpopulation. As described herein, the subpopulation of ES cells expressing Zscan4 can further be identified by co-expression of one or more genes disclosed herein, including AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1.

Totipotent cell: Refers to a cell that can form an entire organism autonomously. Only a fertilized egg (oocyte) possesses this ability (stem cells do not).

Transgenic animal: A non-human animal, usually a mammal, having a non-endogenous (heterologous) nucleic acid sequence present as an extrachromosomal element in a portion of its cells or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal according to methods well known in the art. A "transgene" is meant to refer to such heterologous nucleic acid, such as, heterologous nucleic acid in the form of an expression construct (such as for the production of a "knock-in" transgenic animal) or a heterologous nucleic acid that upon insertion within or adjacent to a target gene results in a decrease in target gene expression (such as for production of a "knock-out" transgenic animal).

Transfecting or transfection: Refers to the process of introducing nucleic acid into a cell or tissue. Transfection can be achieved by any one of a number of methods, such as, but not limited to, liposomal-mediated transfection, electroporation and injection.

Trim43 (tripartite motif-containing protein 43): A gene identified herein as exhibiting morula-specific expression during embryonic development. The nucleotide and amino acid sequences of Trim43 are provided herein as SEQ ID NO: 32 and SEQ ID NO: 33, respectively.

Zscan4: A group of genes identified herein as exhibiting 2-cell embryonic stage and ES cell-specific expression. In the mouse, the term "Zscan4" refers to a collection of genes including three pseudogenes (Zscan1-ps1, Zscan4-ps2 and Zscan4-ps3) and six expressed genes (Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e and Zscan4f). As used herein, Zscan4 also includes human ZSCAN4. Zscan4 refers to Zscan4 polypeptides and Zscan4 polynucleotides encoding the Zscan4 polypeptides.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

III. Overview of Several Embodiments

Disclosed herein are Zscan4 polypeptides and polynucleotides encoding these polypeptides, which are of use in inhibiting differentiation and increasing proliferation of cells, such as stem cells, including embryonic stem cells. Stem cells, especially ES cells in the undifferentiated condition, were previously considered to be a relatively homogenous cell population. However, described herein is the unique expression of Zscan4 in a subpopulation of stem cells, which establishes the presence of a unique cell population among undifferentiated ES cells and provides the means to identify and isolate these cells. Also described herein is the identification of nine genes co-expressed with Zscan4 in the undifferentiated ES cell subpopulation. These genes include AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1. Further described herein is the identification of Trim43 as a gene exhibiting morula-specific gene expression.

It is disclosed herein that Zscan4 is specifically expressed during the 2-cell embryonic stage and in a subpopulation of embryonic stem cells. There is a genus of Zscan4-related genes, including three pseudogenes (Zscan4-ps1, Zscan4-ps2 and Zscan4-ps3) and six expressed genes (Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e and Zscan4f). The Zscan4 genus also includes human ZSCAN4. It is further disclosed herein that AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1 are co-expressed with Zscan4 during embryonic development. Like Zscan4, during embryonic development, these genes are expressed most abundantly at the 2-cell stage.

Methods are provided herein for inhibiting differentiation of a stem cell comprising increasing the expression of Zscan4 in the stem cell. As described herein, the use of Zscan4 includes the use of any Zscan4 gene, including Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e, Zscan4f and human ZSCAN4. In some embodiments, the Zscan4 gene is at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to Zscan4c (SEQ ID NO: 19), Zscan4d (SEQ ID NO: 21) or Zscan4f (SEQ ID NO: 25). In another embodiment, the Zscan4 gene comprises SEQ ID NO: 60.

Increasing expression of Zscan4 in a cell, such as a stem cell, can be achieved according to any number of methods well known in the art. In one embodiment, increasing expression of Zscan4 in a stem cell comprises transfecting the stem cell with a nucleotide encoding Zscan4 operably linked to a promoter. The promoter can be any type of promoter, including a constitutive promoter or an inducible promoter. In one embodiment, the stem cells are transfected with a vector comprising the nucleotide sequence encoding Zscan4 operably linked to the promoter. The vector can be any type of vector, such as a viral vector or a plasmid vector. In one embodiment, the Zscan4 nucleotide sequence is at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to Zscan4c (SEQ ID NO: 19), Zscan4d (SEQ ID NO: 21) or Zscan4f (SEQ ID NO: 25). In another embodiment, the Zscan4 nucleotide sequence comprises SEQ ID NO: 60.

In one embodiment of the methods described herein, inhibiting differentiation of the stem cell increases viability of the stem cells. In another embodiment, inhibiting differentiation of the stem cell prevents senescence of the stem cell. As described herein, the stem cell can be any type of stem cell, including, but not limited to, an embryonic stem cell, an embryonic germ cell, a germline stem cell or a multipotent adult progenitor cell.

Also provided herein is a method of promoting blastocyst outgrowth of an embryonic stem cell, comprising increasing the expression of Zscan4 in the embryonic stem cell, thereby promoting blastocyst outgrowth of the embryonic stem cell. Promoting blastocyst outgrowth can include increasing the efficiency of outgrowth or increasing the number of embryonic stem cells resulting from blastocyst outgrowth. In one embodiment, the method comprises increasing expression of Zscan4 in the cells during the early stages of blastocyst out-

growth, such as prior to proliferation of the stem cells. As described herein, Zscan4 includes any Zscan4 gene, including Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e, Zscan4f, and human ZSCAN4. In one embodiment, the Zscan4 gene is at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to Zscan4c (SEQ ID NO: 19), Zscan4d (SEQ ID NO: 21) or Zscan4f (SEQ ID NO: 25). In another embodiment, the Zscan4 gene comprises SEQ ID NO: 60.

In one embodiment, increasing the expression of Zscan4 in the stem cell comprises transfecting the stem cell with a nucleotide sequence encoding a Zscan4 operably linked to a promoter. The promoter can be any type of promoter, including an inducible promoter or a constitutive promoter. In one embodiment, the cells are transfected with a vector comprising the nucleotide encoding Zscan4 operably linked to a promoter. The vector can be any type of vector, including a viral vector or a plasmid vector.

A method is also provided for identifying a subpopulation of stem cells expressing Zscan4, comprising transfecting the cells with an expression vector comprising a Zscan4 promoter and a reporter gene, wherein expression of the reporter gene indicates Zscan4 is expressed in the subpopulation of stem cells. In one embodiment, the promoter is a Zscan4c promoter. In another embodiment, the Zscan4c promoter includes the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643, 1-3250, or 1-3347 of SEQ ID NO: 28. In another embodiment, the expression vector comprises the nucleic acid sequence set forth as SEQ ID NO: 28. As described herein, the subpopulation of ES cells expressing Zscan4 are in an undifferentiated state. Further provided is a method of identifying the undifferentiated subpopulation of ES cells by detecting expression of one or more Zscan4 co-expressed genes, such as AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1. Detection of expression of these genes can be accomplished using any means well known in the art, such as, for example, RT-PCR, Northern blot or in situ hybridization. Further provided are isolated stem cells identified according to this method.

An isolated expression vector comprising a Zscan4 promoter operably linked to a nucleic acid sequence encoding a heterologous polypeptide is also provided. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In another embodiment, the Zscan4c promoter comprises the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643, 1-3250, or 1-3347 of SEQ ID NO: 28. In another embodiment, the heterologous polypeptide is a marker, enzyme or fluorescent protein. The expression vector can be any type of vector, including, but not limited to a viral vector or a plasmid vector.

Further provided herein is an ES cell line comprising an expression vector comprising a Zscan4 promoter operably linked to a heterologous polypeptide. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In another embodiment, the Zscan4c promoter comprises the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643, 1-3250, or 1-3347 of SEQ ID NO: 28. In another embodiment, the heterologous polypeptide is a marker, enzyme or fluorescent protein. In one example, the fluorescent protein is Emerald.

An isolated expression vector comprising a Trim43 promoter operably linked to a nucleic acid sequence encoding a heterologous polypeptide is also provided. In one embodiment, the Trim43 promoter comprises at least a portion of the nucleic acid sequence set forth as SEQ ID NO: 31. The portion of SEQ ID NO: 31 to be included in the expression vector is at least a portion of SEQ ID NO: 31 that is capable of promoting transcription of the heterologous polypeptide in a cell in which Trim43 is expressed. In some embodiments, the Trim43 promoter sequence is at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO: 31. In another embodiment, the Trim43 promoter comprises SEQ ID NO: 31. In another embodiment, the Trim43 promoter consists of SEQ ID NO: 31. In some embodiments, the heterologous polypeptide is a marker, enzyme or fluorescent protein. In one example the fluorescent protein is Strawberry. The expression vector can be any type of vector, including, but not limited to a viral vector or a plasmid vector.

Further provided herein is an ES cell line containing an expression vector comprising a Trim43 promoter operably linked to a heterologous polypeptide. In one embodiment, the Trim43 promoter comprises at least a portion of the nucleic acid sequence set forth as SEQ ID NO: 31. In some embodiments, the Trim43 promoter sequence is at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO: 31. In another embodiment, the Trim43 promoter comprises SEQ ID NO: 31. In another embodiment, the Trim43 promoter consists of SEQ ID NO: 31. In another embodiment, the heterologous polypeptide is a marker, enzyme or fluorescent protein. In one example, the fluorescent protein is Strawberry.

Provided herein are antibodies specific for Zscan4. In one embodiment, the Zscan4 antibodies specifically recognize Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e, Zscan4f or human ZSCAN4. Also provided are antibodies specific for each Zscan4 co-expressed gene, including antibodies raised against at least a portion of a polypeptide encoded by AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 or Pif1.

Also described herein are transgenic animals harboring a transgene that includes the Zscan4 polynucleotide sequences disclosed herein. Also provided are transgenic animals harboring a transgene that includes polynucleotide sequences of one or more of the Zscan4 co-expressed genes. Such transgenic animals include, but are not limited to, transgenic mice.

Further provided is a transgenic non-human animal comprising a nucleic acid sequence (a transgene) encoding a heterologous polypeptide operably linked to a Zscan4 promoter. In some embodiments, the heterologous polypeptide is a marker, enzyme or fluorescent protein. In one embodiment, the heterologous polypeptide is fluorescent protein. In one example, the fluorescent protein is Emerald. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In another embodiment, the Zscan4c promoter comprises the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643, 1-3250, or 1-3347 of SEQ ID NO: 28.

In another embodiment, the transgenic non-human animal further comprises a nucleic acid sequence encoding a heterologous polypeptide operably linked to a Trim43 promoter. In one embodiment, the Trim43 promoter comprises the nucleic acid sequence set forth as SEQ ID NO: 31. The heterologous polypeptide can be, for example, a marker, enzyme or fluorescent protein. In one embodiment, the heterologous polypeptide is a fluorescent protein. In one example, the fluorescent protein is Strawberry. In some embodiments, the transgenic non-human animal is a transgenic mouse.

Also provided herein are isolated embryonic stem cells obtained from an embryo of the transgenic non-human animal. In one embodiment, the transgenic non-human animal is a transgenic mouse.

IV. Methods of Inducing Differentiation and/or Inhibiting Proliferation of Stem Cells

A method for inhibiting differentiation of a stem cell is disclosed herein. A method for increasing viability and/or inducing proliferation of a stem cell is also disclosed herein. A method is also provided herein for inhibiting senescence of a stem cell. The methods include altering the level of a Zscan4 polypeptide in the cell, thereby inhibiting differentiation and/or inducing proliferation of the cell, and/or inhibiting senescence of the cell. The cell can be in vivo or in vitro.

It is shown herein that inhibiting Zscan4 in embryos blocks the 2- to 4-cell stage embryonic transition. Inhibition of Zscan4 expression also prevents blastocysts from expanding and implanting and prevents the outgrowth of embryonic stem cells from blastocysts. In addition, in embryonic stem cells, Zscan4 expression is only detected in a subpopulation of undifferentiated stem cells. Thus, expression of Zscan4 plays an important role in maintaining ES cells in an undifferentiated state, which is necessary for ES cell viability and proliferation. Zscan4 is also important in allowing outgrowth of ES cells from blastocysts. Therefore, provided herein are methods of increasing expression of Zscan4 in a stem cell to inhibit differentiation, increase viability and prevent senescence of a stem cell. The methods provided herein also include increasing expression of Zscan4 to promote blastocyst outgrowth of ES cells.

Expression of Zscan4 can be increased to inhibit differentiation and/or induce proliferation. In one example, expression of Zscan4 is increased as compared to a control. Increased expression includes, but is not limited to, at least a 20% increase in the amount of Zscan4 mRNA or polypeptide in a cell as compared to a control, such as, but not limited to, at least about a 30%, 50%, 75%, 100%, or 200% increase of Zscan4 mRNA or polypeptide. Suitable controls include a cell not contacted with an agent that alters Zscan4 expression, or not transfected with a vector encoding Zscan4, such as a wild-type stem cell. Suitable controls also include standard values. Exemplary Zscan4 amino acid sequences are set forth in the Sequence Listing as SEQ ID NO: 16 (Zscan4a), SEQ ID NO: 18 (Zscan4b), SEQ ID NO: 20 (Zscan4c), SEQ ID NO: 22 (Zscan4d), SEQ ID NO: 24 (Zscan4e), SEQ ID NO: 26 (Zscan4f) and SEQ ID NO: 30 (human ZSCAN4).

Specific, non-limiting examples of Zscan4 polypeptides include polypeptides including an amino acid sequence at least about 80%, 85%, 90%, 95%, or 99% homologous to the amino acid sequence set forth in SEQ ID NO: 16, 18, 20, 22, 24, 26 or 30. In a further embodiment, a Zscan4 polypeptide is a conservative variant of SEQ ID NO: 16, 18, 20, 22, 24, 26 or 30, such that it includes no more than fifty conservative amino acid substitutions, such as no more than two, no more than five, no more than ten, no more than twenty, or no more than fifty conservative amino acid substitutions in SEQ ID NO: 16, 18, 20, 22, 24, 26 or 30. In another embodiment, a Zscan4 polypeptide has an amino acid sequence as set forth in SEQ ID NO: 16, 18, 20, 22, 24, 26 or 30.

Fragments and variants of a Zscan4 polypeptide can readily be prepared by one of skill in the art using molecular techniques. In one embodiment, a fragment of a Zscan4 polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids of the Zscan4 polypeptide. In another embodiment, a fragment of a Zscan4 polypeptide includes a specific antigenic epitope found on a full-length Zscan4. In a further embodiment, a fragment of Zscan4 is a fragment that confers a function of Zscan4 when transferred into a cell of interest, such as, but not limited to, inhibiting differentiation or increasing proliferation of the cell.

One skilled in the art, given the disclosure herein, can purify a Zscan4 polypeptide using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the Zscan4 polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the Zscan4 polypeptide primary amino acid sequences may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein.

One of skill in the art can readily produce fusion proteins including a Zscan4 polypeptide and a second polypeptide of interest. Optionally, a linker can be included between the Zscan4 polypeptide and the second polypeptide of interest. Fusion proteins include, but are not limited to, a polypeptide including a Zscan4 polypeptide and a marker protein. In one embodiment, the marker protein can be used to identify or purify a Zscan4 polypeptide. Exemplary fusion proteins include, but are not limited to, green fluorescent protein, six histidine residues, or myc and a Zscan4 polypeptide.

Polynucleotides encoding a Zscan4 polypeptide are also provided, and are termed Zscan4 polynucleotides. These polynucleotides include DNA, cDNA and RNA sequences which encode a Zscan4. It is understood that all polynucleotides encoding a Zscan4 polypeptide are also included herein, as long as they encode a polypeptide with the recognized activity, such as the binding to an antibody that recognizes a Zscan4 polypeptide, or modulating cellular differentiation or proliferation. The polynucleotides include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence of the Zscan4 polypeptide encoded by the nucleotide sequence is functionally unchanged. A Zscan4 polynucleotide encodes a Zscan4 polypeptide, as disclosed herein. Exemplary polynucleotide sequences encoding Zscan4 are set for in the Sequence Listing as SEQ ID NO: 12 (Zscan4-ps1), SEQ ID NO: 13 (Zscan4-ps2), SEQ ID NO: 14 (Zscan4-ps3), SEQ ID NO: 15 (Zscan4a), SEQ ID NO: 17 (Zscan4b), SEQ ID NO: 19 (Zscan4c), SEQ ID NO: 21 (Zscan4d), SEQ ID NO: 23 (Zscan4e), SEQ ID NO: 25 (Zscan4f) and SEQ ID NO: 29 (human ZSCAN4).

In some embodiments, the Zscan4 polynucleotide sequence is at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to Zscan4c (SEQ ID NO: 19), Zscan4d (SEQ ID NO: 21) or Zscan4f (SEQ ID NO: 25). In another embodiment, the Zscan4 gene comprises SEQ ID NO: 60.

The Zscan4 polynucleotides include recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double-stranded forms of DNA. Also included in this disclosure are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the disclosed Zscan4 polypeptide (e.g., a polynucleotide that encodes SEQ ID NO: 16, 18, 20, 22, 24, 26 or 30) under physiological conditions. The term "selec-

tively hybridize" refers to hybridization under moderately or highly stringent conditions, which excludes non-related nucleotide sequences.

Also contemplated herein is the use of a Zscan4 polynucleotide, or the complement of a Zscan4 polynucleotide, for RNA interference. Fragments of Zscan4 polynucleotides or their complements can be designed as siRNA molecules to inhibit expression of one or more Zscan4 proteins. In one embodiment, the siRNA compounds are fragments of a Zscan4 pseudogene. Methods of preparing and using siRNA are generally disclosed in U.S. Pat. No. 6,506,559, incorporated herein by reference (see also reviews by Milhavet et al., *Pharmacological Reviews* 55:629-648, 2003; and Gitlin et al., *J. Virol.* 77:7159-7165, 2003; incorporated herein by reference). The double-stranded structure of siRNA can be formed by a single self-complementary RNA strand or two complementary RNA strands.

The siRNA can comprise one or more strands of polymerized ribonucleotide, and may include modifications to either the phosphate-sugar backbone or the nucleoside. For example, the phosphodiester linkages of natural RNA can be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure can be tailored to allow specific genetic inhibition while avoiding a general panic response in some organisms which is generated by dsRNA. Likewise, bases can be modified to block the activity of adenosine deaminase.

Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition. Nucleic acid containing a nucleotide sequence identical to a portion of a target sequence can be used for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Sequence identity may be optimized by alignment algorithms known in the art and calculating the percent difference between the nucleotide sequences. Alternatively, the duplex region of the RNA can be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript.

Sequence identity can optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of particular target gene sequence is preferred. Alternatively, the duplex region of the RNA can be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the particular target gene (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. hybridization for 12-16 hours; followed by washing). The length of the identical nucleotide sequences may be at least 20, 25, 50, 100, 200, 300 or 400 bases. A 100% sequence identity between the RNA and Zscan4 is not required to practice the present methods.

For siRNA (RNAi), the RNA can be directly introduced into the cell (such as intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing RNA. Physical methods of introducing nucleic acids include injection of a solution containing the RNA, bombardment by particles covered by the RNA, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes in the presence of the RNA. A viral construct packaged into a viral particle can efficiently introduce an expression construct into the cell can provide transcription of RNA encoded by the expression construct. Other methods known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemical-mediated transport, such as calcium phosphate, and the like. Thus, the RNA may be introduced along with components that perform one or more of the following activities: enhance RNA uptake by the cell, promote annealing of the duplex strands, stabilize the annealed strands, or other-wise increase inhibition of the target gene.

RNA may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell can mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region can be used to transcribe the RNA strand (or strands). RNA may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (for example, T3, T7, SP6). The use and production of expression constructs are known in the art (for example, PCT Publication No. WO 97/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693; and the references cited therein). If synthesized chemically or by in vitro enzymatic synthesis, the RNA can be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA can be used with no or a minimum of purification to avoid losses due to sample processing. The RNA can be dried for storage or dissolved in an aqueous solution. The solution can contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

A polynucleotide encoding Zscan4 can be included in an expression vector to direct expression of the Zscan4 nucleic acid sequence. Thus, other expression control sequences including appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons can be included in an expression vector. Generally expression control sequences include a promoter, a minimal sequence sufficient to direct transcription.

The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells (e.g. an antibiotic resistance cassette). Vectors suitable for use include, but are not limited, to the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988). Generally, the expression vector will include a promoter. The promoter can be inducible or constitutive. The promoter can be tissue specific. Suitable promoters include the thymidine kinase promoter (TK), metallothionein I, polyhedron, neuron specific enolase, thyrosine hyroxylase, beta-actin, or other promoters. In one embodiment, the promoter is a heterologous promoter.

In one example, the polynucleotide encoding Zscan4 is located downstream of the desired promoter. Optionally, an enhancer element is also included, and can generally be located anywhere on the vector and still have an enhancing effect. However, the amount of increased activity will generally diminish with distance.

Expression vectors including a polynucleotide encoding Zscan4 can be used to transform host cells. Hosts can include

isolated microbial, yeast, insect and mammalian cells, as well as cells located in the organism. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art, and can be used to transfect any cell of interest. Where the cell is a mammalian cell, the genetic change is generally achieved by introduction of the DNA into the genome of the cell (i.e., stable) or as an episome. Thus, host cells can be used to produce Zscan4 polypeptides. Alternatively, expression vectors can be used to transform host cells of interest, such as stem cells.

A "transfected cell" is a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding Zscan4. Transfection of a host cell with recombinant DNA may be carried out by conventional techniques as are well known in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such as a stem cell, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding Zscan4, and a second foreign DNA molecule encoding a selectable phenotype, such as neomycin resistance. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). Other specific, non-limiting examples of viral vectors include adenoviral vectors, lentiviral vectors, retroviral vectors, and pseudorabies vectors.

Differentiation can be induced, or proliferation decreased, of any cell, either in vivo or in vitro, using the methods disclosed herein. In one embodiment, the cell is a stem cell, such as, but not limited to, an embryonic stem cell, a germline stem cell or a multipotent adult progenitor cell. In several examples, a Zscan4 polypeptide, or a polynucleotide encoding the Zscan4 polypeptide, is introduced into a stem cell to decrease differentiation and/or increase proliferation.

In one example, the cells are stem cells, such as embryonic stem cells. For example, murine, primate or human cells can be utilized. ES cells can proliferate indefinitely in an undifferentiated state. Furthermore, ES cells are totipotent cells, meaning that they can generate all of the cells present in the body (bone, muscle, brain cells, etc.). ES cells have been isolated from the inner cell mass (ICM) of the developing murine blastocyst (Evans et al., *Nature* 292:154-156, 1981; Martin et al., *Proc. Natl. Acad. Sci.* 78:7634-7636, 1981; Robertson et al., *Nature* 323:445-448, 1986). Additionally, human cells with ES properties have been isolated from the inner blastocyst cell mass (Thomson et al., *Science* 282:1145-1147, 1998) and developing germ cells (Shamblott et al., *Proc. Natl. Acad. Sci. USA* 95:13726-13731, 1998), and human and non-human primate embryonic stem cells have been produced (see U.S. Pat. No. 6,200,806, which is incorporated by reference herein).

As disclosed in U.S. Pat. No. 6,200,806, ES cells can be produced from human and non-human primates. In one embodiment, primate ES cells are isolated "ES medium" that express SSEA-3; SSEA-4, TRA-1-60, and TRA-1-81 (see U.S. Pat. No. 6,200,806). ES medium consists of 80% Dulbecco's modified Eagle's medium (DMEM; no pyruvate, high glucose formulation, Gibco BRL), with 20% fetal bovine serum (FBS; Hyclone), 0.1 mM β-mercaptoethanol (Sigma), 1% non-essential amino acid stock (Gibco BRL). Generally, primate ES cells are isolated on a confluent layer of murine embryonic fibroblast in the presence of ES cell medium. In one example, embryonic fibroblasts are obtained from 12 day old fetuses from outbred mice (such as CF1, available from SASCO), but other strains may be used as an alternative. Tissue culture dishes treated with 0.1% gelatin (type I; Sigma) can be utilized. Distinguishing features of ES cells, as compared to the committed "multipotential" stem cells present in adults, include the capacity of ES cells to maintain an undifferentiated state indefinitely in culture, and the potential that ES cells have to develop into every different cell types. Unlike mouse ES cells, human ES (hES) cells do not express the stage-specific embryonic antigen SSEA-1, but express SSEA-4, which is another glycolipid cell surface antigen recognized by a specific monoclonal antibody (see, e.g., Amit et al., *Devel. Biol.* 227:271-278, 2000).

For rhesus monkey embryos, adult female rhesus monkeys (greater than four years old) demonstrating normal ovarian cycles are observed daily for evidence of menstrual bleeding (day 1 of cycle=the day of onset of menses). Blood samples are drawn daily during the follicular phase starting from day 8 of the menstrual cycle, and serum concentrations of luteinizing hormone are determined by radioimmunoassay. The female is paired with a male rhesus monkey of proven fertility from day 9 of the menstrual cycle until 48 hours after the luteinizing hormone surge; ovulation is taken as the day following the leutinizing hormone surge. Expanded blastocysts are collected by non-surgical uterine flushing at six days after ovulation. This procedure generally results in the recovery of an average 0.4 to 0.6 viable embryos per rhesus monkey per month (Seshagiri et al., *Am J Primatol.* 29:81-91, 1993).

For marmoset embryos, adult female marmosets (greater than two years of age) demonstrating regular ovarian cycles are maintained in family groups, with a fertile male and up to five progeny. Ovarian cycles are controlled by intramuscular injection of 0.75 g of the prostaglandin PGF2a analog cloprostenol (Estrumate, Mobay Corp, Shawnee, Kans.) during the middle to late luteal phase. Blood samples are drawn on day 0 (immediately before cloprostenol injection), and on days 3, 7, 9, 11, and 13. Plasma progesterone concentrations are determined by ELISA. The day of ovulation is taken as the day preceding a plasma progesterone concentration of 10 ng/ml or more. At eight days after ovulation, expanded blastocysts are recovered by a non-surgical uterine flush procedure (Thomson et al., *J Med Primatol.* 23:333-336, 1994). This procedure results in the average production of 1.0 viable embryos per marmoset per month.

The zona pellucida is removed from blastocysts, such as by brief exposure to pronase (Sigma). For immunosurgery, blastocysts are exposed to a 1:50 dilution of rabbit anti-marmoset spleen cell antiserum (for marmoset blastocysts) or a 1:50 dilution of rabbit anti-rhesus monkey (for rhesus monkey blastocysts) in DMEM for 30 minutes, then washed for 5 minutes three times in DMEM, then exposed to a 1:5 dilution of Guinea pig complement (Gibco) for 3 minutes. After two further washes in DMEM, lysed trophoectoderm cells are removed from the intact inner cell mass (ICM) by gentle pipetting, and the ICM plated on mouse inactivated (3000 rads gamma irradiation) embryonic fibroblasts.

After 7-21 days, ICM-derived masses are removed from endoderm outgrowths with a micropipette with direct observation under a stereo microscope, exposed to 0.05% Trypsin-

EDTA (Gibco) supplemented with 1% chicken serum for 3-5 minutes and gently dissociated by gentle pipetting through a flame polished micropipette.

Dissociated cells are re-plated on embryonic feeder layers in fresh ES medium, and observed for colony formation. Colonies demonstrating ES-like morphology are individually selected, and split again as described above. The ES-like morphology is defined as compact colonies having a high nucleus to cytoplasm ratio and prominent nucleoli. Resulting ES cells are then routinely split by brief trypsinization or exposure to Dulbecco's Phosphate Buffered Saline (PBS, without calcium or magnesium and with 2 mM EDTA) every 1-2 weeks as the cultures become dense. Early passage cells are also frozen and stored in liquid nitrogen.

Cell lines may be karyotyped with a standard G-banding technique (such as by the Cytogenetics Laboratory of the University of Wisconsin State Hygiene Laboratory, which provides routine karyotyping services) and compared to published karyotypes for the primate species.

Isolation of ES cell lines from other primate species would follow a similar procedure, except that the rate of development to blastocyst can vary by a few days between species, and the rate of development of the cultured ICMs will vary between species. For example, six days after ovulation, rhesus monkey embryos are at the expanded blastocyst stage, whereas marmoset embryos do not reach the same stage until 7-8 days after ovulation. The rhesus ES cell lines can be obtained by splitting the ICM-derived cells for the first time at 7-16 days after immunosurgery; whereas the marmoset ES cells were derived with the initial split at 7-10 days after immunosurgery. Because other primates also vary in their developmental rate, the timing of embryo collection, and the timing of the initial ICM split, varies between primate species, but the same techniques and culture conditions will allow ES cell isolation (see U.S. Pat. No. 6,200,806, which is incorporated herein by reference for a complete discussion of primate ES cells and their production).

Human ES cell lines exist and can be used in the methods disclosed herein. Human ES cells can also be derived from preimplantation embryos from in vitro fertilized (IVF) embryos. Experiments on unused human IVF-produced embryos are allowed in many countries, such as Singapore and the United Kingdom, if the embryos are less than 14 days old. Only high quality embryos are suitable for ES isolation. Present defined culture conditions for culturing the one cell human embryo to the expanded blastocyst have been described (see Bongso et al., *Hum Reprod.* 4:706-713, 1989). Co-culturing of human embryos with human oviductal cells results in the production of high blastocyst quality. IVF-derived expanded human blastocysts grown in cellular co-culture, or in improved defined medium, allows isolation of human ES cells with the same procedures described above for non-human primates (see U.S. Pat. No. 6,200,806).

Precursor cells can also be utilized with the methods disclosed herein. The precursor cells can be isolated from a variety of sources using methods known to one skilled in the art. The precursor cells can be of ectodermal, mesodermal or endodermal origin. Any precursor cells which can be obtained and maintained in vitro can potentially be used in accordance with the present methods. Such cells include cells of epithelial tissues such as the skin and the lining of the gut, embryonic heart muscle cells, and neural precursor cells (Stemple and Anderson, 1992, Cell 71:973-985).

In one example, the cells are mesenchymal progenitor cells. Mesenchymal progenitors give rise to a very large number of distinct tissues (Caplan, J. Orth. Res 641-650, 1991). Mesenchymal cells capable of differentiating into bone and cartilage have also been isolated from marrow (Caplan, J. Orth. Res. 641-650, 1991). U.S. Pat. No. 5,226,914 describes an exemplary method for isolating mesenchymal stem cells from bone marrow.

In other examples, the cells are epithelial progenitor cells or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, Meth. Cell Bio. 21A:229, 1980). In stratified epithelial tissue such as the skin, renewal occurs by mitosis of precursor cells within the germinal layer, the layer closest to the basal lamina. Precursor cells within the lining of the gut provide for a rapid renewal rate of this tissue. The cells can also be liver stem cells (see PCT Publication No. WO 94/08598) or kidney stem cells (see Karp et al., *Dev. Biol.* 91:5286-5290, 1994).

In one non-limited example, neuronal precursor cells are utilized. Undifferentiated neural stem cells differentiate into neuroblasts and glioblasts which give rise to neurons and glial cells. During development, cells that are derived from the neural tube give rise to neurons and glia of the CNS. Certain factors present during development, such as nerve growth factor (NGF), promote the growth of neural cells. Methods of isolating and culturing neural stem cells and progenitor cells are well known to those of skill in the art (Hazel and Muller, 1997; U.S. Pat. No. 5,750,376). Methods for isolating and culturing neuronal precursor cells are disclosed, for example, in U.S. Pat. No. 6,610,540.

V. Zscan4 and Trim43 Promoter Sequences

A Zscan4 promoter or a Trim43 promoter can be included in an expression vector to direct expression of a heterologous nucleic acid sequence. Other expression control sequences including appropriate enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons can be included with the Zscan4 or Trim43 promoter in an expression vector. Generally the promoter includes at least a minimal sequence sufficient to direct transcription of a heterologous nucleic acid sequence. In several examples, the heterologous nucleic acid sequence encodes a polypeptide. However, the heterologous nucleic acid can be any RNA sequence of interest, such as an inhibitory RNA.

Expression vectors typically contain an origin of replication as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use include, but are not limited to the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988).

In one example, an enhancer is located upstream of the Zscan4 or Trim43 promoter, but enhancer elements can generally be located anywhere on the vector and still have an enhancing effect. However, the amount of increased activity will generally diminish with distance. Additionally, two or more copies of an enhancer sequence can be operably linked one after the other to produce an even greater increase in promoter activity.

Generally, an expression vector includes a nucleic acid sequence encoding a polypeptide of interest. A polypeptide of interest can be a heterologous polypeptide, such as a polypeptide that affects a function of the transfected cell. Polypeptides of interest include, but are not limited to, polypeptides that confer antibiotic resistance, receptors, oncogenes, and neurotransmitters. A polypeptide of interest can also be a marker polypeptide, which is used to identify a cell of interest. Marker polypeptides include fluorescent polypeptides, enzymes, or antigens that can be identified using conventional

molecular biology procedures. For example, the polypeptide can be a fluorescent marker (such as green fluorescent protein, Emerald (Invitrogen, Carlsbad, Calif.), Strawberry (Clontech, Mountain View, Calif.), *Aequoria victoria*, or *Discosoma* DSRed); an antigenic marker (such as human growth hormone, human insulin, human HLA antigens); a cell-surface marker (such as CD4, or any cell surface receptor); or an enzymatic marker (such as lacZ, alkaline phosphatase). Techniques for identifying these markers in host cells include immunohistochemistry and fluorescent microscopy, and are well known in the art.

RNA molecules transcribed from an expression vector need not always be translated into a polypeptide to express a functional activity. Specific non-limiting examples of other molecules of interest include antisense RNA molecules complementary to an RNA of interest, ribozymes, small inhibitory RNAs, and naturally occurring or modified tRNAs.

Expression vectors including a Zscan4 or Trim43 promoter can be used to transform host cells. Hosts can include isolated microbial, yeast, insect and mammalian cells, as well as cells located in the organism. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art, and can be used to transfect any cell of interest. Where the cell is a mammalian cell, the genetic change is generally achieved by introduction of the DNA into the genome of the cell (stable integration). However, the vector can also be maintained as an episome.

A "transfected cell" is a host cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule including a Zscan4 promoter element. Transfection of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences including the Zscan4 promoter, and a second foreign DNA molecule encoding a selectable phenotype, such as neomycin resistance. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). Other specific, non-limiting examples of viral vectors include adenoviral vectors, lentiviral vectors, retroviral vectors, and pseudorabies vectors.

In one embodiment described in the Examples below, an expression vector comprising a Zsan4 promoter sequence operably linked to a heterologous polypeptide is used to identify cells that express Zscan4. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In some embodiments, the Zscan4c promoter comprises Zsan4c exon and/or intron sequence. The heterologous protein is typically a marker, an enzyme, or a fluorescent protein. In one embodiment, the heterologous protein is green fluorescent protein (GFP), or a variant of GFP, such as Emerald.

Provided herein is a method of identifying a subpopulation of stem cells expressing Zscan4. In one embodiment, the subpopulation is identified by transfecting the stem cells with an expression vector, wherein the expression vector comprises a Zscan4 promoter sequence and a reporter gene. In one embodiment, the Zscan4 promoter is a Zscan4c promoter. In another embodiment, the Zscan4c promoter comprises the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643, 1-3250, or 1-3347 of SEQ ID NO: 28.

The reporter gene can be any type of identifiable marker, such as an enzyme or a fluorescent protein. In one embodiment, the reporter gene is GFP or a variant of GFP, such as Emerald. Expression of the reporter gene indicates the cell expresses Zscan4. Methods of detecting expression of the reporter gene vary depending upon the type of reporter gene and are well known in the art. For example, when a fluorescent reporter is used, detection of expression can be achieved by fluorescence activated cell sorting or fluorescence microscopy. Identification of a subpopulation of stem cells expressing Zscan4 can be achieved with alternative methods, including, but not limited to, using antibodies specific for Zscan4 or by in situ hybridization. In one embodiment, the subpopulation of ES cells expressing Zscan4 is identified by detecting expression of one or more Zscan4 co-expressed genes, including AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1.

Also described herein is an expression vector comprising a Trim43 promoter sequence operably linked to a heterologous polypeptide. The heterologous protein is typically a marker, an enzyme, or a fluorescent protein. In one embodiment, the heterologous protein is the fluorescent protein Strawberry. In some embodiments, the Trim43 promoter sequence is at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO: 31. In another embodiment, the Trim43 promoter comprises SEQ ID NO: 31. In another embodiment, the Trim43 promoter consists of SEQ ID NO: 31.

Also provided herein are isolated ES cells comprising the Zscan4 or Trim43 expression vectors described herein. In one embodiment, the ES cells are a stable cell line.

VI. Transgenic Animals

The Zscan4 polynucleotide sequences disclosed herein can also be used in the production of transgenic animals such as transgenic mice, as described below. Transgenic animals can also be produced that contain polynucleotide sequences of one or more Zscan4 co-expressed genes, including AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1.

In one embodiment, a non-human animal is generated that carries a transgene comprising a nucleic acid encoding Zscan4 operably linked to a promoter. Specific promoters of use include, but are not limited to, a tissue specific promoter such as, but not limited to, an immunoglobulin promoter, a neuronal specific promoter, or the insulin promoter. Specific promoters of use also include a constitutive promoter, such as, but not limited to, the thymidine kinase promoter or the human β-globin minimal, or an actin promoter, amongst others. The Zscan4 promoter can also be used.

In another embodiment, the transgenic non-human animal carries a transgene comprising a nucleic acid encoding a heterologous peptide, such as a marker, enzyme or fluorescent protein, operably linked to a Zscan4 promoter. In one example, the Zscan4 promoter is a Zscan4c promoter, or a portion thereof. In another embodiment, the Zscan4c promoter comprises the nucleic acid sequence set forth as nucleotides 1-2540 of SEQ ID NO: 28, such as nucleotides 1-2643,

1-3250, or 1-3347 of SEQ ID NO: 28. In one example, the heterologous peptide is the fluorescent protein Emerald.

In another embodiment, the transgenic non-human animal carries a transgene comprising a nucleic acid encoding a heterologous peptide, such as a marker, enzyme or fluorescent protein, operably linked to a Trim43 promoter. In one example, the Trim43 promoter comprises the nucleotide sequence of SEQ ID NO: 31, or a portion thereof. The portion of SEQ ID NO: 31 to be included in the expression vector is at least a portion of SEQ ID NO: 31 that is capable of promoting transcription of the heterologous polypeptide in a cell in which Trim43 is expressed. In some embodiments, the Trim43 promoter sequence is at least 70%, at least 80%, at least 90%, at least 95% or at least 99% identical to SEQ ID NO: 31. In another embodiment, the Trim43 promoter comprises SEQ ID NO: 31. In another embodiment, the Trim43 promoter consists of SEQ ID NO: 31. In one example, the heterologous peptide is the fluorescent protein Strawberry.

In another embodiment, the transgenic non-human animal carries two transgenes, a transgene comprising the Zscan4 promoter linked to a nucleic acid sequence encoding a heterologous peptide, and a transgene comprising the Trim43 promoter linked to a nucleic acid sequence encoding a heterologous peptide, as described above. In some cases, the transgenic non-human animal is a mouse comprising the Zscan4 promoter transgene and the Trim43 promoter transgene. In one specific example, the heterologous polypeptide operably linked to the Zscan4 promoter sequence is the fluorescent protein Emerald and the heterologous polypeptide operably linked to the Trim43 promoter sequence is the fluorescent protein Strawberry. This mouse is referred to as a "rainbow" mouse (see Example 10 below).

Embryos obtained from transgenic "rainbow" animals exhibit green color at the late 2-cell stage and red color at the 4-cell to morula stages (with strongest expression at the morula stage). The expression of these colors at the proper timing and intensity indicates the progress of a correct genetic program, and thus, can be used as indicators of proper development of preimplantation embryos. These embryos have a variety of applications, including, but not limited to development of optimized culture media for human embryos for in vitro fertilization (IVF); training of technicians and clinicians in the IVF clinic and research laboratories; testing of chemical compounds and drugs for embryo toxicity; and as indicators of successful nuclear reprogramming for nuclear transplantation/cloning procedures.

The nucleic acid sequences described herein can be introduced into a vector to produce a product that is then amplified, for example, by preparation in a bacterial vector, according to conventional methods (see, for example, Sambrook et al., *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Press, 1989). The amplified construct is thereafter excised from the vector and purified for use in producing transgenic animals.

Any transgenic animal can be of use in the methods disclosed herein, provided the transgenic animal is a non-human animal. A "non-human animal" includes, but is not limited to, a non-human primate, a farm animal such as swine, cattle, and poultry, a sport animal or pet such as dogs, cats, horses, hamsters, rodents, or a zoo animal such as lions, tigers or bears. In one specific, non-limiting example, the non-human animal is a transgenic animal, such as, but not limited to, a transgenic mouse, cow, sheep, or goat. In one specific, non-limiting example, the transgenic animal is a mouse. In a particular example, the transgenic animal has altered proliferation and/or differentiation of a cell type as compared to a non-transgenic control (wild-type) animal of the same species.

A transgenic animal contains cells that bear genetic information received, directly or indirectly, by deliberate genetic manipulation at the subcellular level, such as by microinjection or infection with a recombinant virus, such that a recombinant DNA is included in the cells of the animal. This molecule can be integrated within the animal's chromosomes, or can be included as extrachromosomally replicating DNA sequences, such as might be engineered into yeast artificial chromosomes. A transgenic animal can be a "germ cell line" transgenic animal, such that the genetic information has been taken up and incorporated into a germ line cell, therefore conferring the ability to transfer the information to offspring. If such offspring in fact possess some or all of that information, then they, too, are transgenic animals.

Transgenic animals can readily be produced by one of skill in the art. For example, transgenic animals can be produced by introducing into single cell embryos DNA encoding a marker, in a manner such that the polynucleotides are stably integrated into the DNA of germ line cells of the mature animal and inherited in normal Mendelian fashion. Advances in technologies for embryo micromanipulation permit introduction of heterologous DNA into fertilized mammalian ova. For instance, totipotent or pluripotent stem cells can be transformed by microinjection, calcium phosphate mediated precipitation, liposome fusion, retroviral infection or other means. The transformed cells are then introduced into the embryo, and the embryo then develops into a transgenic animal. In one non-limiting method, developing embryos are infected with a retrovirus containing the desired DNA, and a transgenic animal is produced from the infected embryo.

In another specific, non-limiting example, the appropriate DNA(s) are injected into the pronucleus or cytoplasm of embryos, preferably at the single cell stage, and the embryos are allowed to develop into mature transgenic animals. These techniques are well known. For instance, reviews of standard laboratory procedures for microinjection of heterologous DNAs into mammalian (mouse, pig, rabbit, sheep, goat, cow) fertilized ova include: Hogan et al., *Manipulating the Mouse Embryo*, Cold Spring Harbor Press, 1986; Krimpenfort et al., *Bio/Technology* 9:86, 1991; Palmiter et al., *Cell* 41:343, 1985; Kraemer et al., *Genetic Manipulation of the Early Mammalian Embryo*, Cold Spring Harbor Laboratory Press, 1985; Hammer et al., *Nature* 315:680, 1985; Purcel et al., *Science* 244:1281, 1986; U.S. Pat. No. 5,175,385; U.S. Pat. No. 5,175,384.

VII. Antibodies

A Zscan4 polypeptide or a fragment or conservative variant thereof can be used to produce antibodies which are immunoreactive or specifically bind to an epitope of a Zscan4. Polyclonal antibodies, antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are included. In one embodiment, the Zscan4 antibodies recognize all Zscan4 proteins, including Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e, Zscan4f and human ZSCAN4. In another embodiment, the antibodies specifically recognize only one Zscan4 protein. As used herein, the ability of an antibody to specifically a particular Zscan4 protein means that the antibody detects expression of one Zscan4 protein, but none of the other Zscan4 proteins. In an alternative embodiment, the antibodies recognize two or more different Zscan4 proteins. For example, a Zscan4 antibody may recog-

nize only the Zscan4 proteins comprising a SCAN domain (e.g., Zscan4c, Zscan4d, Zscan4f). Or, a Zscan4 antibody may recognize only the Zscan4 proteins comprising the zinc finger domains, but lacking the SCAN domain (e.g., Zscan4a, Zscan4b, Zscan4e).

Antibodies can also be raised against one or more proteins encoded by genes identified herein as Zscan4 co-expressed genes. Thus, in some embodiments, a polypeptide encoded by AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 or Pif1, or a fragment or conservative variant thereof, can be used to produce antibodies which are immunoreactive or specifically bind to an epitope of the polypeptide.

In addition, antibodies can be generated that specifically bind Trim43. In one embodiment, a Trim43 polypeptide, or a fragment or conservative variant thereof, can be used to produce antibodies which are immunoreactive or specifically bind to an epitope of Trim43.

The preparation of polyclonal antibodies is well known to those skilled in the art. See, for example, Green et al., "Production of Polyclonal Antisera," in: *Immunochemical Protocols*, pages 1-5, Manson, ed., Humana Press, 1992; Coligan et al., "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," in: *Current Protocols in Immunology*, section 2.4.1, 1992.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* 256: 495, 1975; Coligan et al., sections 2.5.1-2.6.7; and Harlow et al. in: *Antibodies: a Laboratory Manual*, page 726, Cold Spring Harbor Pub., 1988. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes et al., Purification of Immunoglobulin G (IgG), in: *Methods in Molecular Biology*, Vol. 10, pages 79-104, Humana Press, 1992.

Methods of in vitro and in vivo multiplication of monoclonal antibodies are well known to those skilled in the art. Multiplication in vitro may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally supplemented by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, thymocytes or bone marrow macrophages. Production in vitro provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large-scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication in vivo may be carried out by injecting cell clones into mammals histocompatible with the parent cells, such as syngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Antibodies can also be derived from a subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in PCT Publication No. WO 91/11465, 1991; and Losman et al., *Int. J. Cancer* 46:310, 1990.

Alternatively, an antibody that specifically binds a Zscan4 polypeptide can be derived from a humanized monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:3833, 1989. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones et al., *Nature* 321:522, 1986; Riechmann et al., *Nature* 332:323, 1988; Verhoeyen et al., *Science* 239:1534, 1988; Carter et al., *Proc. Natl. Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer et al., *J. Immunol.* 150:2844, 1993.

Antibodies can be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas et al., in: *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 119, 1991; Winter et al., *Ann. Rev. Immunol.* 12:433, 1994. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, Calif.).

In addition, antibodies can be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al., *Nature Genet.* 7:13, 1994; Lonberg et al., *Nature* 368:856, 1994; and Taylor et al., *Int. Immunol.* 6:579, 1994.

Antibodies include intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with their antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

(5) Single chain antibody (SCA), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988). An epitope is any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Pat. No. 4,036,945 and U.S. Pat. No. 4,331,647, and references contained therein; Nisonhoff et al., *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman et al., *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan et al. at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent (Inbar et al., *Proc. Natl. Acad. Sci. U.S.A.* 69:2659, 1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, e.g., Sandhu, supra. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are known in the art (see Whitlow et al., *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird et al., *Science* 242:423, 1988; U.S. Pat. No. 4,946,778; Pack et al., *Bio/Technology* 11:1271, 1993; and Sandhu, supra).

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick et al., *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 106, 1991).

Antibodies can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from substantially purified polypeptide produced in host cells, in vitro translated cDNA, or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

Polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (see, for example, Coligan et al., Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first mono-clonal antibody.

Binding affinity for a target antigen is typically measured or determined by standard antibody-antigen assays, such as competitive assays, saturation assays, or immunoassays such as ELISA or RIA. Such assays can be used to determine the dissociation constant of the antibody. The phrase "dissociation constant" refers to the affinity of an antibody for an antigen. Specificity of binding between an antibody and an antigen exists if the dissociation constant (K_D=1/K, where K is the affinity constant) of the antibody is, for example <1 μM, <100 nM, or <0.1 nM. Antibody molecules will typically have a K_D in the lower ranges. K_D=[Ab−Ag]/[Ab][Ag] where [Ab] is the concentration at equilibrium of the antibody, [Ag] is the concentration at equilibrium of the antigen and [Ab−Ag] is the concentration at equilibrium of the antibody-antigen complex. Typically, the binding interactions between antigen and antibody include reversible noncovalent associations such as electrostatic attraction, Van der Waals forces and hydrogen bonds.

Effector molecules, e.g., therapeutic, diagnostic, or detection moieties can be linked to an antibody that specifically binds Zscan4, using any number of means known to those of skill in the art. Exemplary effector molecules include, but not limited to, radiolabels, fluorescent markers, or toxins (e.g. *Pseudomonas* exotoxin (PE), see "*Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet*," Thorpe et al., "Monoclonal Antibodies in Clinical Medicine," Academic Press, pp. 168-190, 1982; Waldmann, *Science,* 252: 1657, 1991; U.S. Pat. No. 4,545,985 and U.S. Pat. No. 4,894,443, for a discussion of toxins and conjugation). Both covalent and noncovalent attachment means may be used. The procedure for attaching an effector molecule to an antibody varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; e.g., carboxylic acid (COOH), free amine (—NH_2) or sulfhydryl (—SH) groups, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule. Alternatively, the antibody is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of linker molecules such as those available from Pierce Chemical Company, Rockford, Ill. The linker can be

any molecule used to join the antibody to the effector molecule. The linker is capable of forming covalent bonds to both the antibody and to the effector molecule. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody and the effector molecule are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (e.g., through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In some circumstances, it is desirable to free the effector molecule from the antibody when the immunoconjugate has reached its target site. Therefore, in these circumstances, immunoconjugates will comprise linkages that are cleavable in the vicinity of the target site. Cleavage of the linker to release the effector molecule from the antibody may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, label (e.g. enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies, one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the invention to the particular features or embodiments described.

EXAMPLES

The characterization of Zscan4 is disclosed herein. Zscan4 is shown herein to exhibit transient and specific expression at the late 2-cell embryonic stage and in embryonic stem cells. Without being bound by theory, Zscan4 is the only gene that is exclusively expressed during the first wave of de novo transcription, zygotic genome activation.

Zscan4 was identified from a cDNA clone derived from ES cells (clone number C0348C03) and subsequently sequenced by the Mammalian Gene Collection project (Gerhard et al. *Genom Res.* 14:2121-2127, 2004). The cDNA sequence, deposited under Genbank Accession No. BC050218 (SEQ ID NO: 11), comprised 2292 bp organized into 4 exons encoding a protein of 506 amino acids. As described in the Examples below, using this cDNA clone as a probe, a high level of Zscan4 transcript was detected in late 2-cell stage embryos. Since the original cDNA was isolated from ES cells, RT-PCR was performed on RNAs derived from late 2-cell stage embryos and the amplification product was sequenced, as described in the Examples below. The amplified sequence was 2268 bp in length and like the cDNA isolated from ES cells, encoded a protein of 506 amino acids. Analysis of the nucleotide and amino acid sequences of the cDNA clones isolated from ES cells and late 2-cell embryos showed they were two different, but similar genes.

As described in the Examples below, nine Zscan4 gene copies were identified in the mouse genome. Three copies are pseudogenes and were designated Zscan4-ps1 (SEQ ID NO: 12), Zscan4-ps2 (SEQ ID NO: 13) and Zscan4 ps3 (SEQ ID NO: 14), according to the convention of mouse gene nomenclature. The remaining six gene copies are transcribed and encode ORFs, thus they were named Zscan4a (SEQ ID NOs: 15 and 16), Zscan4b (SEQ ID NOs: 17 and 18), Zscan4c (SEQ ID NOs: 19 and 20), Zscan4d (SEQ ID NOs: 21 and 22), Zscan4e (SEQ ID NOs: 23 and 24) and Zscan4f (SEQ ID NOs: 25 and 26). Zscan4c, Zscan4d and Zscan4f encode proteins of 506 amino acids, while Zscan4a, Zscan4b and Zscan4e encode shorter proteins of 360, 195 and 195 amino acids, respectively. A polypeptide comprising any of the amino acid sequences set forth as SEQ ID NOs: 16, 18, 20, 22, 24, 26 or 30, or a polynucleotide encoding these polypeptides, are of use in the methods disclosed herein. A polynucleotide encoding a Zscan4 pseudogene set forth as SEQ ID NOs: 12, 13 or 14 are also of use in the methods disclosed herein.

Analysis of the expression levels of Zscan4 demonstrated that expression of each of the six Zscan4 genes could be detected in ES cells with Zscan4c being the predominant transcript. Zscan4d was the predominant transcript in 2-cell stage embryos; however, low levels of Zscan4a Zscan4e and Zscan4f could also be detected. These findings are consistent with the origin of each cDNA clone since Zscan4c was derived from the ES cell cDNA library and Zscan4d was derived from the 2-cell embryo cDNA library. Furthermore, expression of Zscan4 was not detected in blastocysts (including the inner cell mass) or early blastocyst outgrowth. After approximately six days of outgrowth, Zscan4 expression was detected in a subpopulation of undifferentiated ES cells.

It is shown herein that expression of Zscan4 is temporally regulated and its expression or lack of expression at different embryonic stages is critical to proper development. As described in the Examples below, inhibition of Zscan4 expression in embryos blocked the 2- to 4-cell embryonic transition, prevented blastocysts from expanding, prevented blastocysts from implanting and prevented proliferation of ES cells from blastocyst outgrowths.

Also described herein is the development of a mouse ES cell line expressing a heterologous protein, Emerald, under the control of a Zscan4 promoter. Further described is the identification of nine Zscan4 co-expressed genes exhibiting 2-cell stage specific expression.

Also shown herein is the identification of Trim43 as a gene exhibiting expression during the 4-cell to morula embryonic stages, with the highest level of expression observed at the morula stage. Also described herein is the development of a transgenic mouse, which comprises two transgenes, the first comprising Emerald operably linked to the Zscan4c promoter and the second comprising Strawberry operably linked to the Trim43 promoter.

Example 1

Materials and Methods

Identification and Cloning of the Mouse Zscan4d Gene

Using DNA microarray data of mouse preimplantation embryos (Hamatani et al., *Dev. Cell* 6:117-131, 2004), Zscan4d gene was identified for its specific expression in 2-cell embryos. A corresponding cDNA clone (no. C0348C03; R1 ES cells, 129 strain; Genbank Accession No. BC050218, SEQ ID NO: 11) was identified in the mouse cDNA collection described previously (Sharov et al., *PLoS Bio.* 1:E74, 2003). Based on this full-length cDNA sequence, a primer pair (5'-cctccctgggcttcttggcat-3', SEQ ID NO: 1; 5'-agctgccaaccagaaagacactgt-3', SEQ ID NO: 2) was designed and used to PCR-amplify the full-length cDNA sequence of this gene from 2-cell embryos (B6D2F1 mouse). In brief, mRNA was extracted from 2-cell embryos and treated with DNAase (DNA-free, Ambion). The mRNA was annealed with an oligo-dT primer and reverse-transcribed into cDNA with ThermoScript Reverse Transcriptase (Invitrogen). A full-length cDNA clone was PCR-amplified with

Ex Taq Polymerase (Takara Minis Bio, Madison, Wis.), purified with the Wizard SV Gel and PCR Clean-Up System (Promega Biosciences, San Luis Obispo, Calif.), cloned into a pENTR plasmid vector with the Directional TOPO Cloning Kit (Invitrogen), and completely sequenced using BigDye Terminator kit (PE Applied Biosystems, Foster City, Calif.) and DyeEX 96 Kit (Qiagen Valencia, Calif.) on ABI 3100 Genetic Analyzer (PE Applied Biosystems). The sequence is set forth herein as SEQ ID NO: 21).

The WU-BLAST (available online) and UCSC genome browser were used to obtain Zscan4 orthologs in the human genome sequence. Open reading frames (ORFs) were deduced by ORF finder (available online from the National Center for Biotechnology Information) and protein domains were identified by Pfam HMM database (available online). Orthologous relationships were assessed with the phylogenetic tree of amino acid sequences determined by a sequence distance method and the Neighbor Joining (NJ) algorithm (Saitou and Nei, 1987) using Vector NTI software (Invitrogen, Carlsbad, Calif.).

All gene names and gene symbols were consulted with and approved by the mouse gene nomenclature committee.

Southern Blot Analysis

Southern blot analysis was carried out to validate the genome sequence of the Zscan4 locus assembled using individual BAC clone sequences downloaded from the public database (RPCI-23 and RPCI-24 BAC libraries: C57BL/6J strain). A probe containing exon 3 was designed and amplified from mouse DNA extracted from ES cells (C57BL/6) using a primer pair (5'-gcattcctacataccaatta-3', SEQ ID NO: 3; 5'-gatttaatttagctgggctg-3', SEQ ID NO: 4). The PCR product was purified using GFX PCR DNA and Gel band purification kit (GE Healthcare). Fifteen μg of mouse genomic DNA extracted from ES cells (BL6.9 line derived from C57BL/6 strain) was digested overnight with restriction enzymes (MspI, TaqI, and MspI/TaqI, see FIG. **3**B), fractionated on a 1% (w/v) agarose gel, transferred and immobilized onto nitrocellulose membranes. Blots were hybridized with random-primed ^{32}P-labeled DNA probes under standard conditions. Membranes were subjected to 3 washes of 30 min each (2×SSC/0.1% (w/v) SDS at room temperature, 0.5× SSC/0.1% (w/v) SDS at 42° C., and 0.1×SSC/0.1% (w/v) SDS at room temperature) and autoradiographed for 48 hours at −80° C.

Measurement of Gene Expression Levels

cDNAs from ES cells (129.3 ES cells purchased from the Transgenic Core Laboratory of the Johns Hopkins University School of Medicine, Baltimore, Md.) and 2-cell embryos (B6D2F1 mice) were synthesized. Zscan4 cDNA fragments were amplified using a Zscan4-specific primer pair (Zscan4_For:5'-cagatgccagtagacaccac-3', SEQ ID NO: 5; Zscan4_Rev 5'-gtagatgttccttgacttgc-3', SEQ ID NO: 6), which were 100%-matched to all Zscan4 paralogs. These cDNA fragments were sequenced using the following primers: Zscan4_For, 5'-cagatgccagtagacaccac-3', SEQ ID NO: 5; Zscan4__400Rev, 5'-ggaagtgttatagcaattgttc-3', SEQ ID NO: 7; Zscan4_Rev, 5'-gtagatgttccttgacttgc-3', SEQ ID NO: 6; and Zscan4__300Rev, 5'-gtgttatagcaattgttcttg-3', SEQ ID NO: 8. Electropherograms of these sequences were used to calculate the relative expression levels of nine paralogous copies of Zscan4 in the following manner. Based on sequence information of transcripts (either predicted from the genome sequence or determined by sequencing cDNA clones), nucleotide positions were identified where one or a few paralogous copies can be distinguished based on the nucleotide mismatches. The phred base calling program (version 0.020425.c (Ewing et al., *Genome Res.* 8:175-185, 1998)) was used to obtain the amplitudes of all four bases in the electropherogram for those nucleotide sites. After subtracting the noise level (i.e., the average of amplitudes of the bases that are not present in any of the nine paralogous copies), the amplitudes of each base (A, T, G, C) were obtained. The expression levels of each of the paralogous copies were calculated by the least square fitting, which found the expression levels that are most consistent with all mismatched nucleotide positions.

Collection and Manipulation of Embryos

Four- to six-week old B6D2F1 mice were superovulated by injecting 5 IU pregnant mare serum gonadotropin (PMS; Sigma, St Louis, Mo., USA) and 5 IU human chorionic gonadotropin (HCG; Sigma) after 46-47 h (Protocol#220MSK-Mi approved by the National Institute on Aging Animal Care and Use Committee). Unfertilized eggs were harvested at 21 h post-HCG according to the standard method (Nagy et al., 2003, "Manipulation of the Mouse Embryo, A Laboratory Manual," Cold Spring Harbor Laboratory Press, New York). After removing cumulus cells by incubation in M2 medium (MR-015-D) supplemented with bovine testicular hyaluronidase (HY, 0.1% (w/v), 300 Umg-1), unfertilized eggs were thoroughly washed, selected for good morphology and collected. Fertilized eggs (1-cell embryos) were also harvested from mated superovulated mice in the same way as unfertilized eggs. Fertilized eggs (1-cell embryos) were cultured in synthetic oviductal medium enriched with potassium (KSOMaa MR-121-D) at 37° C. in an atmosphere of 5% CO2. For the embryo transfer procedure, 3.5 d.p.c. blastocysts were transferred into the uteri of 2.5 d.p.c. pseudopregnant ICR female mice.

To synchronize in vitro embryo development, embryos with two pronuclei (PN) were selected. When some of these 1-cell stage embryos started to cleave, the early 2-cell stage embryos were selected and transferred to another microdrop culture. The early 2-cell stage embryos were cultured until some of them started 2^{nd} cleavage and the embryos that were still at the 2-cell stage were collected. These embryos were synchronized at the late 2-cell stage.

DNA was microinjected into embryos according to the following procedures.

(1) Pronuclear injection: Plasmid vectors constitutively expressing a siRNA against mouse Zscan4 were constructed by inserting the following target sequences in a pRNAT-U6.1/Neo vector (GenScript Corp., Scotch Plains, N.J., USA), shZscan4 (gagtgaattgctttgtgtc, SEQ ID NO: 9) and siControl (randomized 21-mer, agagacatagaatcgcacgca, SEQ ID NO: 10). This vector contains a green fluorescence protein (GFP) marker under a cytomegalovirus (CMV) promoter. For RNA interference experiments, 1-2 pl (2-3 ng/μl) of a linearized vector DNA (shZscan4 or shControl) was microinjected into the male pronucleus of zygotes. A plasmid vector constitutively expressing the Zscan4d gene was constructed by cloning the CDS of Zscan4d into a plasmid pPyCAGIP (Chambers et al., *Cell* 113:643-655, 2003). For overexpression experiments, 1-2 pl (2-3 ng/l) of plasmid DNA (Zscan4d-inserted or no insert pPyCAGIP vector) linearized by ScaI was microinjected into the male pronucleus of zygotes.

(2) Cytoplasmic injection: Transient RNA interference experiments were carried out by microinjecting ~10 pl (5 ng/μl) of oligonucleotide (siZscan4, plus-siZscan4, and siControl) into the cytoplasm of zygotes. The optimal amount of siRNA was determined by testing different concentrations of siRNA (4, 20, and 100 ng/μl).

All siRNAs were resuspended and diluted with the microinjection buffer (Specialty Media). The transfer of cultured blastocysts into pseudopregnant recipients was done according to the standard protocol (Nagy et al., 2003, "Manipulation

of the Mouse Embryo, A Laboratory Manual," Cold Spring Harbor Laboratory Press, New York). All media were purchased from Specialty Media (Phillipsburg, N.J.).

Culture of ES Cells and Blastocyst Outgrowth

A mouse ES cell line (129.3 line derived from strain 129 and purchased from The Transgenic Core Laboratory of the Johns Hopkins University School of Medicine, Baltimore, Md., USA) was first cultured for two passages into a gelatin-coated culture dish in the presence of leukemia inhibitory factor (LIF) to remove contaminating feeder cells. Cells were then seeded on gelatin coated 6-well plates at the density of 1-2×10^5/well (1-2×10^4/cm^2) and cultured for 3 days with complete ES medium (DMEM, 15% FBS; 1000 U/ml ESGRO (mLIF; Chemicon, Temecula, Calif.); 1 mM sodium pyruvate; 0.1 mM NEAA; 2 mM glutamate; 0.1 mM beta-mercapto ethanol and 50 U/50 μg per ml penicillin/streptomycin).

For the outgrowth experiments, blastocysts at 3.5 days post coitum (d.p.c.) were cultured individually in DMEM (Gibco catalog no. 10313-021) supplemented with 15% fetal bovine serum, 15 mM HEPES buffer, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 100 μM nonessential amino acids, 4.5 mM of L-glutamine, and 100 μM of β-mercapto ethanol on gelatinized chamber slides at 37° C. in 5% CO2.

Whole Mount In Situ Hybridization (WISH)

A plasmid DNA (clone C0348C03) was digested with SalI/NotI and transcribed in vitro into digoxigenin-labeled antisense and sense probe as control. Embryos obtained from young (7 weeks old) B6D2F1a mice were fixed in 4% paraformaldehyde and used to perform whole mount in situ hybridization (WISH) according to the previously described protocol. WISH was also carried out on cultured ES cells according to the same protocol (Yoshikawa et al., *Gene Expr. Patterns* 6:213-224, 2006).

Quantitative Reverse Transcriptase PCR

Embryos for quantitative reverse transcriptase (qRT)-PCR experiments were collected as described above and harvested at 23, 43, 55, 66, 80 and 102 hours post-hCG for 1-cell, early 2 cell, late 2-cell, 4-cell, 8-cell, morula and blastocyst embryos, respectively. Three subsets of 10 synchronized and intact embryos were transferred in PBT 1X (PBS supplemented 0.1% Tween X20) and stored in liquid nitrogen. These pools of embryos were mechanically ruptured by a freeze/thaw and directly used as a template for cDNA preparations. The Ovation system (NuGen technologies, San Carlos, Calif., USA) was used to synthesize cDNAs from each pool. The cDNAs were then diluted to 1:25 in a total of 1000 μl and 2 μl was used as a template for qPCR. The qPCR was performed on the ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif., USA) as previously described (Falco et al., *Reprod. Biomed. Online* 13:394-403, 2006) and data were normalized by Chuk and H2afz with the ΔΔCt method (Falco et al., *Reprod. Biomed. Online* 13:394-403, 2006; Livak and Schmittgen, *Methods* 25:402-408, 2001). Embryos subjected to RNA interference experiments were analyzed in the same way as described above for the normal preimplantation embryos

Example 2

Identification of 2-Cell-Specific Genes During Preimplantation Development

After fertilization, the maternal genetic program governed by maternally stored RNAs and proteins must be switched to the embryonic genetic program governed by de novo transcription, called zygotic genome activation (ZGA), from the newly-formed zygotic genome (DePamphilis et al., "Activation of Zygotic Gene Expression" In Advances in Developmental Biology and Biochemistry, Vol. 12, pp. 56-84, Elsevier Science B.V., 2002; Latham and Schultz, *Front Biosci.* 6:D748-759, 2001). The ZGA is one of the first and most critical events in animal development. Earlier reports have established that the ZGA begins during the 1-cell stage (Aoki et al., *Dev. Biol.* 181:296-307, 1997) (Nothias et al., *J. Biol. Chem.* 270:22077-22080, 1995; Ram and Schultz, *Dev. Biol.* 156:552-556, 1993). However, global gene expression profiling by DNA microarray analysis has recently revealed that nearly all genes identified for their increase of expression at the 1-cell stage were insensitive to alpha-amanitin treatment, which blocks RNA polymerase II (Hamatani et al., *Dev. Cell* 6:117-131, 2004; Zeng and Schultz, *Dev. Biol.* 283:40-57, 2005). Thus, these studies not only identified many ZGA genes, but also revealed that de novo transcription of the zygotic genome begins during the 2-cell stage of mouse preimplantation development (Hamatani et al., *Dev. Cell* 6:117-131, 2004; Zeng and Schultz, *Dev. Biol.* 283:40-57, 2005). Furthermore, it has been shown that the major burst of ZGA does not occur at the early 2-cell stage, but during the late 2-cell stage (Hamatani et al., *Dev. Cell* 6:117-131, 2004).

Arrest of development at the 2-cell stage has been reported for the loss-of-function mutants of Mater/Nalp5 (Tong et al., *Nat. Genet.* 26:267-268, 2000), Mhr6a/Ube2a (Roest et al., *Mol. Cell. Biol.* 24:5485-5495, 2004) and Brg1/Smarca4 (Bultman et al., *Genes Dev.* 20:1744-1754, 2006). Although the timing of the developmental arrest coincides with that of the ZGA, these genes are expressed during oogenesis and stored in oocytes, but are not transcribed in the 2-cell stage. Therefore, these maternal effect genes are not suitable for the study of the ZGA. Previously the ZGA has been studied using either exogenous plasmid-borne reporter genes Nothias et al., *J. Biol. Chem.* 270:22077-22080), or endogenous, but rather ubiquitously expressed genes, such as Hsp70.1 (Christians et al., 1995), eIF-4C (Davis et al., *Dev. Biol.* 174:190-201, 1996), Xist (Zuccotti et al., *Mol. Reprod. Dev.* 61:14-20, 2002) and other genes (DePamphilis et al., "Activation of Zygotic Gene Expression" In Advances in Developmental Biology and Biochemistry, Vol. 12, pp. 56-84, Elsevier Science B.V., 2002). Although TEAD-2/TEF-4 (Kaneko et al., *Development* 124:1963-1973, 1997) and Pou5f1/Oct4 (Palmieri et al., *Dev. Biol.* 166:259-267, 1994) are considered as transcription factors selectively expressed at ZGA (DePamphilis et al., "Activation of Zygotic Gene Expression" In Advances in Developmental Biology and Biochemistry, Vol. 12, pp. 56-84, Elsevier Science B.V., 2002), these genes are known to be expressed in cells other than 2-cell embryos. It is thus important to identify and study individual ZGA genes, especially the genes expressed exclusively at the 2-cell stage.

Global gene expression profiling of preimplantation embryos was previously carried out and a group of genes was identified that showed transient spike-like expression in the 2-cell embryo (Hamatani et al., *Dev. Cell* 6:117-131, 2004). By examining the expression of these genes in the public expressed sequence tag (EST) database (NCBI/NIH), a novel gene was identified represented by only 29 cDNA clones out of 4.7 million mouse ESTs. These cDNA clones have been isolated from cDNA libraries derived from ES cells and preimplantation embryos. Furthermore, the previous DNA microarray data showed that the expression of this gene is detected in ES cells, but not in embryonal carcinoma (EC) cells (F9 and P19), trophoblast stem (TS) cells, or neural stem/progenitor (NS) cells (Aiba et al., *Stem Cells* 24:889-895, 2006).

One of the cDNA clones derived from ES cells (clone number C0348C03; (Sharov et al., *PLoS Biol.* 1:E74, 2003)) was completely sequenced by the Mammalian Gene Collection (MGC) project (Genbank Accession No. BC050218; SEQ ID NO: 11 (Gerhard et al., *Genome Res.* 14:2121-2127, 2004)). Whole mount in situ hybridization (WISH) using this cDNA clone as a probe detected high level of transcripts in late 2-cell embryos (FIG. **1**A). The transcript was not detected in unfertilized eggs and embryos in other preimplantation stages including 3-cell embryos, suggesting a high specificity of gene expression at the late 2-cell stage and a relatively short half-life of the transcripts. Quantitative reverse-transcriptase PCR (qRT-PCR) analysis confirmed the WISH results (FIG. **1**B). Previous microarray analysis showed that the expression of this gene at the late 2-cell stage was suppressed in embryos treated with α-amanitin (a blocker of RNA pol II-based transcription) (Hamatani et al., *Dev. Cell* 6:117-131, 2004), confirming that this gene is transcribed de novo during the major burst of ZGA. The transient expression pattern was observed in both in vitro cultured embryos and freshly isolated in vivo embryos (Hamatani et al., *Dev. Cell* 6:117-131, 2004).

Example 3

Structure and Expression of Zscan4 Paralogous Genes

The full-length cDNA sequence (BC050218; SEQ ID NO: 11) of 2292 bp was organized into 4 exons, encoding a protein of 506 amino acids (FIG. **2**A). Because this cDNA clone was isolated from a cDNA library made from ES cells (Sharov et al., *PLoS Biol.* 1:E74, 2003), another cDNA clone was isolated by performing RT-PCR on RNAs isolated from late 2 cell-stage embryos and completely sequenced (SEQ ID NO: 21). This 2268 bp cDNA clone encoded a protein of 506 amino acids. DNA sequence and protein sequences clearly showed that these two cDNAs (SEQ ID NOs: 11 and 21) were two different genes with close similarity. Domain prediction analysis revealed a SCAN (Leucine Rich Element) domain and four zinc finger domains at the N- and C-terminal ends, respectively (FIG. **2**B). A hypothetical human ortholog—zinc finger and SCAN domain containing 4 (ZSCAN4) was also identified that shares 45% of amino acid sequence similarity with the high conservation in SCAN (50%) and zinc finger domains (59%) (FIG. **7**).

Alignment of full-length cDNA sequences (SEQ ID NOs: 11 and 21) to the mouse genome sequence (mm7) revealed multiple hits in the proximal region of chromosome 7, the syntenic region of human ZSCAN4 (FIG. **8**). One notable feature of this genome region was repetitions of a very similar sequence segment. The sequences of each copy of Zscan4 and the surrounding region were very similar to each other, leaving the assembled genome sequences of this region less accurate than those of other regions. To understand the genome structure of this region better, individual BAC clone sequences were manually reassembled from this region into ~850 kb genome sequence contigs (FIG. **3**A). Because it was difficult to find a hybridization probe or oligonucleotides to distinguish each copy, restriction enzymes were used that can distinguish small sequence differences among gene copies. Southern blot analysis was carried out by digesting C57BL/6J mouse genomic DNAs with TaqI alone, MspI alone, or TaqI/MspI (FIGS. **3**B and C). All the detected DNA fragments confirmed nine paralogous Zscan4 genes predicted in the assembled genome sequences.

The full-length cDNA sequence (BC050218; SEQ ID NO: 11) was then aligned to the assembled genome sequence and nine gene copies were found, all of which had multi-exon gene organizations (FIGS. **2**, **3**A). Three gene copies were apparently pseudogenes as no evidence was found that they were transcribed based on available EST information and sequencing analysis of RT-PCR products. Therefore, the genes were named Zscan4 ps1 (SEQ ID NO: 12), Zscan4-ps2 (SEQ ID NO: 13), and Zscan4-ps3 (SEQ ID NO: 14), according to the convention of mouse gene nomenclature. Because the remaining 6 gene copies were transcribed and encoded ORFs, they were named Zscan4a (SEQ ID NO: 15), Zscan4b (SEQ ID NO: 17), Zscan4c (SEQ ID NO: 19), Zscan4d (SEQ ID NO: 21), Zscan4e (SEQ ID NO: 23) and Zscan4f (SEQ ID NO: 25). Three of the these genes, Zscan4a, Zscan4b, and Zscan4e, encoded ORFs of 360, 195 and 195 amino acids, respectively, which included the SCAN domain, but not the four zinc finger domains (FIG. **2**B).

The remaining three genes, Zscan4c, Zscan4d and Zscan4f, encoded full-length ORFs (506 amino acids). The main features of these genes are summarized in FIG. **3**A. Zscan4c corresponds to the cDNA clone isolated from ES cells (C0348C03; Genbank Accession No. BC050218; Gm397; SEQ ID NO: 11). Zscan4d corresponds to the cDNA clone isolated from 2-cell embryos (SEQ ID NO: 21). Zscan4f corresponds to a gene predicted from the genome sequence (Genbank Accession No. XM_145358; SEQ ID NO: 27). Similarities of both ORFs and mRNAs between these three genes were very high (FIG. **7**). Thus, it is most likely that these three genes have the same function. To measure the expression levels of each paralog, DNA sequences of the nine Zscan4 paralogs were analyzed by the Clustal X multiple-sequence alignment program, which showed the presence of sequence differences specific to each paralog. To examine the expression levels of each gene in 2-cell embryos and ES cells, cDNA fragments amplified by RT-PCR from 2-cell embryos and ES cells were sequenced. The expression level of each paralog were estimated based on the amplitudes of each nucleotide at polymorphic sites. The results are summarized in FIG. **3**A. In 2-cell embryos, Zscan4d was a predominant transcript (90%). In contrast, in ES cells, Zscan4c was a predominant transcript (40%), although Zscan4f was a lesser, but significant transcript (24%). These results were consistent with the origin of each cDNA clone; Zscan4c was derived from the ES cell cDNA library, whereas Zscan4d was derived from the 2-cell embryo library.

Example 4

Function of Zscan4 in Preimplantation Development

As a first step to characterize the function of Zscan4 genes, the studies focused on preimplantation development. Initially a possibility to carry out a standard gene targeting strategy was explored, but it was difficult for the following three reasons. First, sequences of Zscan4 paralogs and surrounding genomic regions are too similar to design targeting constructs for specific genes. Second, it is highly likely that Zscan4d$^{-/-}$ phenotype can be compensated functionally by other Zscan4 paralogs, because in addition to predominantly-expressed Zscan4d, at least 3 other similar copies (Zscan4a, Zscan4e, and Zscan4f) were also transcribed in 2-cell embryos. Third, the presence of other predicted genes, though not annotated as genes yet, within ~850 kb Zscan4 locus makes a strategy to delete the entire Zscan4 locus less attractive. Therefore, siRNA technology was used. Although RNAi and siRNA technology has been successfully used for blocking the expression of specific genes in preimplantation embryos (Kim et al., *Biochem. Biopys. Res. Commun.* 296:1372-1377,

2002; Stein et al., *Dev. Biol.* 286:464-471, 2005), widely-recognized off-target effects are generally a major concern (Jackson et al., *Rna* 12:1179-1187, 2006; Scacheri et al., *Proc. Natl. Acad. Sci. U.S.A.* 101:1892-1897, 2004; Semizarov et al., *Proc. Natl. Acad. Sci. U.S.A.* 100:6347-6352, 2003). To increase the confidence of the effects by siRNA against Zscan4, the siRNA experiments were carried out by three independent siRNA technologies, an oligonucleotide-based siRNA (denoted here siZscan4 and obtained from Invitrogen); a vector-based shRNA (denoted here shZscan4 and obtained from Genscript); and a mixture of oligonucleotide siRNAs (denoted here plus-siZscan4 and obtained from Dharmacon) (FIGS. **4**A, B). Oligonucleotide sequences used for siZscan4, shZscan4, plus-siZscan4 matched 100% with cDNA sequences of Zscan4a, Zscan4b, Zscan4c, Zscan4d, Zscan4e and Zscan4f, except for shZscan4 with 2 bp mismatches with Zscan4b and Zscan4e (FIG. **4**A, B).

A shZscan4 vector was microinjected into the male pronucleus of zygotes at 21-23 hours after the hCG injection and embryos were observed during preimplantation development (FIGS. **4**C and D). At 61 hours post-hCG, when the majority (58.8%) of shControl-injected embryos have already reached the 4-cell stage, the majority (78.8%) of shZscan4-injected embryos remained at the 2-cell stage. By 98 hours post-hCG, when the majority (70.0%) of shControl-injected embryos have reached blastocyst stage, the majority (52.5%) of shZscan4-injected embryos reached only morula stage. A significant reduction (~95%) of Zscan4 RNA levels was confirmed by the qRT-PCR analysis (FIG. **4**E). Taken together, these results indicate that the development of shZscan4-injected embryos was delayed for about 24 hrs between the 2- and 4-cell stages, followed by progression to the later stages at a speed comparable to that of shControl-injected embryos. Essentially the same results were obtained using two different siRNA technologies: siZscan4 (FIG. **9**) and plus-siZscan4 (FIG. **10**).

siZscan4-injected embryos formed normal looking early blastocysts (3.5 d.p.c.), but often failed to form expanded blastocysts (4.5 d.p.c.; 45% of siZscan4-injected embryos versus 6% of siControl-injected embryos; FIG. **9**B). To test whether these blastocysts had any compromise even at 3.5 d.p.c., shZscan4-injected blastocysts were transferred to the uterus of pseudo-pregnant mice. None of the shZscan4-injected blastocysts implanted, whereas most shControl-injected embryos implanted (Table 1). In vitro blastocyst outgrowth experiments determined that cells of shZscan4-injected blastocysts failed to proliferate in culture (Table 1). These results clearly demonstrated that the transient expression of Zscan4 at the late 2-cell stage is required for the development of proper blastocysts.

TABLE 1

Blastocyst outgrowth (A) and post-implantation development (B) of embryos received pronuclear injection of shZscan4 or shControl

A Blastocyst Outgrowth	Number of tested blastocysts	Number of successful outgrowth
shZscan4	16	0
shControl	17	7

TABLE 1-continued

Blastocyst outgrowth (A) and post-implantation development (B) of embryos received pronuclear injection of shZscan4 or shControl

B Embryo Transfer	Number of blastocysts transferred to pseudo-pregnant mother	Number of pups born
shZscan4	8	0
shControl	10	4

*A shZscan4 or shControl vector was microinjected into the male pronucleus of zygotes at 21-23 hours after the hCG injection. Early blastocysts (3.5 d.p.c.) formed from these embryos were subjected to tests of blastocysts outgrowth (A) and embryo transfer (B). In the outgrowth assay, the presence of proliferating cells after 6 days in culture was considered as successful outgrowth.

The notion that the reduction of Zscan4 expression level delays the development of preimplantation embryos at the 2-cell stage was further supported by the fact that when shZscan4 was injected into one of the blastomeres of early 2-cell stage embryos, ~28% of embryos became 3-cell embryos (FIG. **5**A). One blastomere that received shZscan4 injection remained as a 2-cell blastomere, whereas the other blastomere cleaved into two smaller blastomeres with the size of 4-cell blastomeres (FIG. **5**D). Subsequently, these embryos (24%) became unevenly cleaved embryos, typically 5-cell embryos, with one 2-cell-sized blastomere and four 8-cell-sized blastomeres (FIG. **5**B, E). These embryos eventually formed blastocyst-like structures, but they seemed to be the mixtures of blastocyst-like cell mass and morula-like cell mass, which was often GFP-positive, a marker for shRNA-injected blastomere (FIG. **5**C, F, G). In contrast, when shControl was injected into one of the blastomeres at the early 2-cell stage, nearly all embryos cleaved normally (FIGS. **5**A, B, C).

To investigate the effect of prolonged Zscan4d expression on preimplantation development, Zscan4d was overexpressed by microinjecting a Zscan4d-expressing plasmid into the male pronucleus of zygotes. Although the Zscan4d plasmid-injected embryos showed a rate of development similar to control plasmid-injected embryos, the former blastocysts failed to produce the outgrowth (Table 2A) and failed to implant (Table 2B). The results suggest that the timely down-regulation of Zscan4d is also important for the proper development of blastocysts.

TABLE 2

Blastocyst outgrowth (A) and post-implantation development (B) of embryos received pronuclear injection of a Zscan4d-expressing plasmid or a control plasmid

A Blastocyst Outgrowth	Number of tested blastocysts	Number of successful outgrowth
Zscan4d-expressing plasmid	10	2
Control plasmid	15	11

B Embryo Transfer	Number of blastocysts transferred to pseudo-pregnant mother	Number of pups
Zscan4d-expressing plasmid	10	0
Control plasmid	14	5

*A plasmid vector constitutively expressing Zscan4d gene or control empty vector was microinjected into the male pronucleus of zygotes at 21-23 hours after the hCG injection. Early blastocysts (3.5 d.p.c.) formed from these embryos were subjected to the same tests as described in Table 1.

Example 5

Analysis of Zscan4 Expression Using the Whole Mount In Situ Hybridization (WISH)

One intriguing aspect of the expression pattern of Zscan4 is the exclusive expression in late 2-cell embryos and ES cells. This appears to be counter-intuitive, because ES cells are derived from the ICM and many genes that are expressed in ES cells are also expressed in the ICM (e.g., Yoshikawa et al., *Gene Expr. Patterns* 6:213-224, 2006). Therefore the expression of Zscan4 in blastocysts, blastocyst outgrowth, and ES cells was examined using WISH. The results demonstrated that the expression of Zscan4 was not detected anywhere in blastocysts, including the ICM and the early blastocyst outgrowth (FIG. **6**A). However, the expression of Zscan4 began to be detected in a small fraction of cells by the day 6 of the outgrowth. Surprisingly, the strong expression of Zscan4 was detected in only a small fraction of ES cells in undifferentiated colonies. In contrast, the expression of Pou5f1 (Oct3/4), a well-known marker for pluripotency, was detected in the ICM of blastocysts, a large fraction of the cells in the blastocyst outgrowth, and the majority of ES cells in undifferentiated colonies (FIG. **6**A). Due to the close similarity of cDNA sequences, each Zscan4 paralog could not be distinguished by WISH, but the expression analysis by sequencing RT-PCR products mentioned above indicates that Zscan4c and Zscan4f were the genes detected in the subpopulation of the cells in blastocyst outgrowth and ES cells by WISH.

Example 6

Zscan4 Promoter Expression Vector

As described in previous Examples herein, Zscan4 expression is only detected in a subpopulation of undifferentiated ES cells. In order to identify this subpopulation of ES cells, and to identify any other cell expressing Zscan4, an expression plasmid was developed which comprises a Zscan4c promoter sequence and the Emerald reporter gene (a variant of green fluorescent protein). The components and orientation of the expression vector are illustrated in FIG. **11**. The sequence of the Zscan4c promoter-Emerald expression vector is set forth as SEQ ID NO: 28. The nucleotide ranges of SEQ ID NO: 28 of the components of the expression vector are provided in Table 3.

TABLE 3

Zscan4c Promoter-Emerald Expression Vector

Component	Nucleotides of SEQ ID NO: 28
Zscan4c promoter	1-3347
TATA box	2483-2489
Zscan4c exon 1	2541-2643
Zscan4c intron 1	2644-3250
Zscan4c exon 2 (partial)	3251-3347
Emerald start codon	3398-3400
Emerald reporter gene	3398-4117
TK poly A signal	4132-4403
EM7 promoter	5257-5323
Blasticidin selection gene	5330-5722
SV40 polyA signal	5880-6010

Mouse ES cells were transfected with the Zscan4c promoter expression vector and analyzed by fluorescence activated cell sorting to identify Emerald-positive cells and Emerald-negative cells. If Zscan4 is expressed in a cell, it is Emerald-positive. The results show approximately 3-5% of mouse ES cells express Zscan4 (FIG. **12**).

Sorted cells were collected and analyzed by quantitative real time PCR (qPCR) for expression of Zscan4c and Pou5f1 (also known as Oct3, Oct4, Oct3/4), a well known marker for pluripotency. As shown in FIG. **12**, Pou5f1 is expressed at the same level in both Emerald-positive and Emerald-negative cells, whereas Zscan4c is more highly expressed in Emerald-positive cells than in Emerald-negative cells. The data indicate that the Zscan4c promoter sequence used in this vector can reproduce the expression of endogenous Zscan4c gene, and thus the Zscan4c promoter-Emerald expression vector can be used to purify Zscan4-expressing cells. The data also indicate that both Zscan4-expressing cells and non-expressing cells retain the pluripotency-marker Pou5f1 expression, thus this subpopulation of ES cells cannot be identified by a standard pluripotency marker.

Example 7

Mouse ES Cell Line Expressing Emerald Under Control of the Zscan4 Promoter

A mouse ES cell line was established in which the Zscan4c promoter expression vector described in Example 6 was stably incorporated into the cells. The ES cell line expresses Emerald under control of the Zscan4c promoter. After transfecting a linearlized plasmid DNA into mouse ES cells, the cells were cultured in the presence of the selectable marker (blasticidin). The blasticidin-resistant ES cell clones were isolated and used for further analysis.

As described herein, Zscan4 is only expressed in a subpopulation of undifferentiated ES cells (approximately 3-5% of ES cells). Accordingly, the ES cell line incorporating the Zscan4 promoter expression vector exhibits expression in only a small percentage, approximately three percent, of cells.

Example 8

Identification of Nine Genes Co-Expressed with Zscan4 in a Sub-Population of ES Cells

Using the mouse ES cell line stably transfected with the Zscan4c promoter (as described in Example 7), DNA microarray analysis was performed to compare gene expression patterns of Emerald(+) and Emerald(−) cells. Emerald (+) and Emerald(−) cells were sorted by FACS and total RNAs were isolated from each cell population. These RNAs were labeled and hybridized to the NIA-Agilent 44K DNA microarray (Agilent Technologies).

Nine genes were identified as being co-expressed with Zscan4: AF067063, Tcstv1/Tcstv3, Tho4, Arginase II, BC061212 and Gm428, Eif1a, EG668777 and Pif1. In situ hybridization was performed to confirm expression of these genes in mouse ES cells. The 2-cell embryo-specific expression profiles of six of these genes (AF067063, Tcstv3, Tho4, Arginase II, BC061212 or Gm428) are shown in FIGS. **13**A-G.

Example 9

Trim43 is Specifically Expressed in 4-Cell to Morula Stage Embryos

To identify genes that are specifically expressed at the 8-cell and morula stages, publicly available EST frequency

data (TIGR Mouse Gene Index; MGI Library Expression Search; NIA Mouse Gene Index (Sharov et al., *PLoS Bio.* 1:E74, 2003)) and microarray data from mouse preimplantation embryos (Hamatani et al., *Dev. Cell* 6 (1):117-31, 2004) were used. After selecting candidate genes, quantitative RT-PCR analysis was carried out to confirm the specific expression pattern of Trim43 (tripartite motif-containing protein 43).

Trim43 expression was detected beginning at the 4-cell embryonic stage and peaked at the morula stage. A low level of Trim43 expression was detected in blastocysts. The function of the Trim43 protein is unknown. The nucleotide and amino acid sequences of Trim43 are provided herein as SEQ ID NO: 32 and SEQ ID NO: 33, respectively. The nucleic acid sequence of the Trim43 promoter is provided herein as SEQ ID NO: 31.

Example 10

Transgenic "Rainbow" Mouse

As described herein, an expression vector comprising a Zscan4c promoter operably linked to a first heterologous polypeptide (Emerald) and an expression vector comprising a Trim43 promoter operably linked to a second heterologous polypeptide (Strawberry), have been generated. A transgenic mouse (a "rainbow" mouse) can be generated which incorporates both of these expression constructs.

A 7155 base pair DNA fragment containing the Insulator-Zscan4 promoter-emerald and TK polyA and a 8672 base pair DNA fragment containing the Insulator-Trim43 promoter-Strawberry are co-injected into the pronucleus of fertilized mouse eggs (B6C3X B6).

Embryos obtained from the rainbow mouse will exhibit green color (as a result of expression of Emerald) at the late 2-cell stage, and red color (due to expression of Strawberry) from the 4-cell stage to the morula stage (with peak expression at the morula stage). The expression of Emerald and Strawberry at the appropriate stage of embryonic development indicates proper development of the embryo. Thus, these embryos will be useful for a number of research and clinical purposes. For example, embryos obtained from the rainbow mouse can be used to develop optimized culture conditions for embryos, which can be applied to human embryos used in the IVF clinic. In addition, these embryos can be used to test chemical compounds or drugs for toxicity to the embryo. The embryos can also be used as indicators of successful nuclear reprogramming for nuclear transplantation procedures.

This disclosure provides methods of inhibiting differentiation of stem cells and promoting blastocyst outgrowth of ES cells. The disclosure further provides a Zscan4 promoter sequence and methods of use, including identification of a subpopulation of stem cells expressing Zscan4. It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described invention. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 4

gatttaattt agctgggctg 20

<210> SEQ ID NO 5
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 5

cagatgccag tagacaccac 20

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 6

gtagatgttc cttgacttgc 20

<210> SEQ ID NO 7
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 7

ggaagtgtta tagcaattgt tc 22

<210> SEQ ID NO 8
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 8

gtgttatagc aattgttctt g 21

<210> SEQ ID NO 9
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: siRNA

<400> SEQUENCE: 9

gagtgaattg ctttgtgtc 19

<210> SEQ ID NO 10
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: siRNA

<400> SEQUENCE: 10

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agagacatag aatcgcacgc a 21

<210> SEQ ID NO 11
<211> LENGTH: 2292
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 11

gacacacagt gcctccctgg gcttcttggc atcacccttg aagttcaccg gagaaagcag 60

tgaggtggag gaataggtaa actttccttc ctagtggtct tgaatgtctt ttacagtaca 120

tccatcaact gttagcattt tcgtaaagtc acaaaacaga tattaaacta ctatagttga 180

atctttcaca ccattgtcac cacaatggct tcacagcagg caccagcaaa agaccttcag 240

accaacaatt tagagtttac tccaactgat agttctggtg tgcagtgggc agaagacatc 300

tctaactcac caagtgctca gctaaacttt tccccaagta acaatggctg ctgggcaact 360

caggagctgc aaagtctctg gaagatgttc aactcctggt tgcagccaga aaagcagact 420

aaggagcaga tgatttctca actggtcttg gagcagtttc tcctcactgg gcactgcaag 480

gacaagtatg ctttgacaga gaagtggaaa gccagtggta gcgatatgag gagattcatg 540

gagagtctga ctgatgagtg cttgaagcct cctgtcatgg tccatgtttc aatgcaagga 600

caagaagccc tcttttctga aaacatgcca ttaaaagaag tcatcaagct tttgaaacaa 660

cagcaatctg caacaaggcc aacaccagat aatgagcaga tgccagtaga caccacacaa 720

gatagattat tggccacagg acaagaaaac agtgaaaatg aatgcaacaa ctcttgtaat 780

gctactgaag caaatgttgg tgaaagctgt agtggaaatg aaatggactc ccttcttatt 840

atccagaaag aacagcaccc tgagcatgaa gaggggaatg ttgtttgtca attccctcat 900

ggtgccagaa gagcaagtca aggcaccccc agtcatcatg tagacttccc gagtgctccg 960

actactgccg atgtccccat ggaggaacaa ccaaaggatt tatccagaga aaacatctct 1020

gaggacaaga acaattgcta taacacttcc agaaatgcag ctactcaagt atatagtggt 1080

gataatattc ccaggaacaa gtcagactcc cttttcatta acaagagaat atatcatcct 1140

gagcctgagg tgggagatat tccttatgga gttcctcagg attctacaag agcaagtcaa 1200

ggaacatcta catgcctgca agagtcactt ggggaatgtt tttctgaaaa cgacccaagg 1260

gaggtaccag ggttgcagtc taggcaagag cagcctatct ctgatcctgt ccttcttggt 1320

aagaatcatg aggcaaactt accatgtgaa agtcatcaaa agagattctg tagagatgcc 1380

aaactataca agtgtgaaga atgttctagg atgttcaaac atgccaggag cctttcatcc 1440

caccagagaa ctcacctgaa taagaagagt gaattgcttt gtgtcacctg tcagaaaatg 1500

ttcaaacgag tctctgaccg ccgcacccat gagatcatac acatgccaga aaagcctttc 1560

aagtgcagca catgtgaaaa gtccttcagc cacaagacca acctgaagtc tcatgagatg 1620

attcacacag gagaaatgcc ttatgtctgt tccctatgta gccgtcgctt tcgccaatca 1680

tccacttacc atcgtcacct gaggaattac cacagatctg actgaactat ctaacatcct 1740

cagcagagac tggtagggct tcagcctcag tatgtcatct tcaaagagag aagaatgttg 1800

caagtaaatt gtactgtccc aataatgata taacatgctt gtggattgcc acttttatgt 1860

tttgttttgt tttttatttt gtgtgtgtgt gtatgtaatt ttttgtctgt atttccatat 1920

ttccacagca taagttatta gaatactttg ctgttaattc ttgagttgct tcttgctttt 1980

agacagtgtc tttctggttg gcagctttat aaacctgtct ttctggcact agagtttcca 2040

aacattttct ggtctccact tttattctct acagtgttct tgacagaagc ctggcattcc 2100

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ctctgacatt ttctacatgt tggggttttc atcccaagtc ttagggttgc aagttaaatg 2160

cattgcctct tcagacatct catgccatgt ctactgctta cagttcaaga atatttctct 2220

acattactag aacgacgttc aaagtggaat aataaataaa taaataatca acaattaaaa 2280

aaaaaaaaaa aa 2292

<210> SEQ ID NO 12
<211> LENGTH: 2288
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

cacagtgcct ccctgggctt cttggcatca cccttaaagt tcactggaga aagaggtgag 60

gtggaggagt aggtaaactt ccctacctag tggtcttgaa tgtcttttat agtacatcca 120

tcaactgtta gcattttcct aaagtcacaa aacagatact aaactgctat agttgaatct 180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca 240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta 300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaattcagg 360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg 420

agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca 480

agtatacttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga 540

gtctgactga tgagtgcttg aagcctccag tcatggtcca tgtttcaatg caaggacaag 600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc 660

aatctgcaac aaggccaaca ccagataatg agcagatgcc agtagacacc acacaagata 720

gattattggc cacaggacaa gaaaacagtg aaaatgaatg caacacctct tgtaatgcta 780

ctgaaggaaa tgttggtgaa agatgtggtg gaaatgaaat ggactccctt cttattatcc 840

agaaagaaca gcaccctgag catgaagagg ggaatgttgt ttgtcgattc cctcatggtg 900

ccagaagagc aagtcaaggc aactctagtc atcatgtaga cttccggagt gctctgactc 960

ctgcggatgt ccccatggag gaacaaccaa aggatttatc cagagaaaac atctctgagg 1020

acaagaacaa ttgctataac acttccagga atgcagctac tcaagtatat agcagtgata 1080

atattcccag gaaaaaaaca gactcccttt ccattaacaa gagaatatat catcctgagc 1140

ctgaggtggg agatattcct tatggagttc ctcatgattc tacaagagca agtcaaggaa 1200

catctacatg cctgcaagag tcacttgggg aatgtttttc tgaaaaagac cctagggagg 1260

taccagggtt ggagtctagg caagaggagc ctatctctga tcctgtcctt cttggtaaga 1320

atcatgaggc aaacttacca tgtgaaagtc atcataagag attccgtaga gatgccaaac 1380

tatacaagtg tgaagaatgt tctaggatgt tcaaacatgc caggagcctt tcatcccacc 1440

agagaactca cctgaataag aagagtgaat tgctttgttt cacctgtcag aaaatgttca 1500

aacgagtctc tgaccgccga acccatgaga tcatacacat gccagaaaag cctttcaagt 1560

gcagcacatg tgaaaagtcc ttcagccaca agaccaacct gaagtctcat gagatgattc 1620

acacaggaga aatgccttat gtctgttccc tatgtagccg tcgctttcgc caatcatcca 1680

cttaccatcg tcacctgagg aattaccaca gatctgactg aactatctaa catccttagc 1740

agagactggt agagcttcag cctcagtatg tcatcttcaa agagagaaga atgttgctac 1800

taaattgtac tttcccaatg atgatataac atgcttgtag agtgccactt ttatgttttg 1860

ttttgttttg ttttgttttg ttttgttttg tgtgtgtgtg tgtgtgtgtg taatttttg 1920

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tctgtatttc catagttcca cagcataagt tattagaata ctttgctgtt aattcttgag 1980

ttgtttcttg cttttaaaca gtggccttct ggttggcagc tttatacacc tgtctttatg 2040

gcattagagt ttccaaacat tttctgatct ccacttttat tctctacagt ggtcctgaca 2100

gaggcctgcc attccctctg acatttttct acctgttggg gttttaatcc acagtcttaa 2160

ggttgcaagt taaatgcatt gccttttcag acatctccca tgtcatgtct actgcttaca 2220

gtatatttct ctacattact agaatgacat tcaaagtgga gtaataaata aataaataat 2280

caacaatt 2288

<210> SEQ ID NO 13
<211> LENGTH: 2273
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcactggaga aagaggtgag 60

gtggaggaat aggtaaactt tccttcctag tggtcttgaa tgtcttttac agtacatcca 120

tcaactgtta gcattttcgt aaagtcacaa aacagatatt aaactactat agttgaatct 180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca 240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta 300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg 360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg 420

agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca 480

agtatgcttt gactgagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga 540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtttcaatg caaggacaag 600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc 660

aatatgcaac aaggccaaca ccagataatg agcagatgcc agtagacacc acacaagata 720

gattattggc cacaggacaa gaaaacagtg aaaatgaatg caacaactct tgtaatgcta 780

ctgaaggaaa tgttggtgaa agctgtagtg gaaatgaaat ggactccctt cttattatcc 840

agaaagaaca gcaccctgag catgaagagg ggaatgttgt ttgtcaattc cctcatggtg 900

ccagaagagc aagtcaaggc acccccagtc atcatgtaga cttcccgagt gttccgacta 960

ctgccgatgt ccccatggag gaacaaccaa aggatttatc cagagaaaac atctctgagg 1020

acaagaacaa ttgctataac acttccagaa atgcagctac tcaagtatat agtggtgata 1080

atattcccag gaacaagtca gactcccttt tcattaacaa gagaatatat catcctgagc 1140

ctgaggtggg agatattcct tatggagttc ctcaggattc tacaagagca agtcaaggaa 1200

catctacatg cctgcaagag tcacttgggg aatgtttttc tgaaaaagac cctagggagg 1260

taccagggtt gcagtctagg caagagcagc ttatctctga tcctgtcctt cttggtaaga 1320

atcatgaggc aaacttacca tgtgaaagtc atcaaaagag attctgtaga gatgccaaac 1380

tatacaagtg tgaagaatgt tctaggatgt tcaaacatgc caggagcctt tcatcccacc 1440

agagaactca cctgaataag aagagtgaat tgctttgtgt cacctgtcag aaaatgttca 1500

aacgagtctc tgaccgccga acccatgaga tcatacacat gccagaaaag cctttcaagt 1560

gcagcacatg tgaaaagtcc ttcagccaca agaccaacct gaagtctcat gagatgattc 1620

acacaggaga aatgccttat gtctgttccc tatgtagccg tcgctttcgc caatcatcca 1680

cttaccatcg tcacctgagg aattaccaca gatctgactg aactatctaa catcctcagc 1740

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agagactggt agggcttcag cctcagtatg tcatcttcaa agagagaaga atgttgcaag 1800

taaattgtac tgtcccaata atgatataac atgcttgtgg attgccactt ttatgttttg 1860

ttttgttttt tattttgtgt gtgtgtgtat gtaatttttt gtctgtattt ccatagttcc 1920

acagcataag ttattagaat actttgctgt taattcttga gttgcttctt gcttttagac 1980

agtgtctttc tggttgacag ctttataaac ctgtctttct ggcactagag tttccaaaca 2040

ttttctgatc tccactttta ttctctacag tgttcttgac agaagcctgg cattccctct 2100

gacatttttc tacatgttgg ggttttcatc ccaagtctta gggttgcaag ttaaatgcat 2160

tgcctcttca gacatctcat gccctgtcta ctgcttacag ttcaagaata tttctctaca 2220

ttactagaac gacattcaaa gtggaataat aaataaataa ataatcaaca att 2273

<210> SEQ ID NO 14
<211> LENGTH: 2273
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

cacagtgcct ccctgggctt cttggcatca ccctagaagt tcactggaga aagaggtgag 60

gtggaggaat aggtaaactt tccttcctag tggtcttgaa tgtcttttac agtacactat 120

cagctgttag cattttccta aagtcacaaa acagatacta aactgctata gttgaatctt 180

tcacaccatt gtcaccacaa tggcttcaca gcaggcacca gcaaaagacc ttcagaccaa 240

caatttagag tttactccaa ctgatagttc tggtgtgcag tgggcagaag acatctctaa 300

ctcaccaagt gctcagctaa acttttcccc aagtaacaat ggctgctggg caactcagga 360

gctgcaaagt ctctggaaga tgttcaactc ctggttgcag ccagaaaagc agactaagga 420

gcagatgatt tctcaactgg tcttggagca gtttctcctc actgggcact gcaaggacaa 480

gtatgctttg acagagaagt ggaaagccag tggtagcgat atgaggagat tcatggagag 540

tctgactgat gagtgcttga agcctcctgt catggtccat gtctcaatgc aaggacaaga 600

agcactcttt tctgaaaaca tgccattaaa agaagtcatc aagcttttga aacaacagca 660

atatgcaaca aggccaacac cagataatga gcagatgcca gtagacacca cacaagatag 720

attattggcc acaggacaag aaaacagtga aaatgaatgc aacaactctt gtaatgctac 780

tgaagcaaat gttggtgaaa gctgtagtgg aaatgaaatg gactcccttc ttatcatcca 840

gaaagaacag caccctgagc atgaagaggg gaatgttgtt cgtcaattcc ctcatggtgc 900

cagaagagca agtcaaggca cccccagtca tcatgtagac atccagagtc ctccgactac 960

tgccgatgtc accatggagg aacaaccaaa ggatttatcc agagaaaaca tctctgagga 1020

caagaacaat tgctataaca cttccaggaa tgcagctact caagtatata gtggtgataa 1080

tattcccagg aacaagtcag actccctttt cattaacaag agaatatatc atcctgagcc 1140

tgaggtggga gatattcctt atggatttcc tcaggattct acaagagcaa gtcaaggaac 1200

atctacatgc ctgcaagagt cacttgggga atgtttttct gaaaaagacc ctagggaggt 1260

accagggttg cagtctaggc aagagcagct tatctctgat cctgtccttc ttggtaagaa 1320

tcatgaggca aacttaccat gtaaaagtca tcaaaagaga ttctgtagag atgccaaact 1380

atacaagtgt gaagaatgtt ctaggatgtt caaacatgcc aggagccttt catcccacca 1440

gaaaactcac ctcaataaga agagtgaatt gctttgtgtc acctgtcaga aaatgttcaa 1500

acgagtctct gaccgccgaa cccatgagat catacacatg ccagaaaagc ctttcaagtg 1560

cagcacatgt gaaaagtcct tcagccacaa gaccaacctg aagtctcatg agatgattca 1620

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cacaggagaa atgccttatg tctgttccct atgtagccgt cgctttcgcc aatcatccac 1680

ttaccatcgt cacctgagga attaccacag atctgactga actatctaac atcctcagca 1740

gagactggta gggcttcagc ctcagtatgt catcttcaaa gagagaagaa tgttgcaagt 1800

aaattgtact gtcccaataa tgatataaca tgcttgtgga ttgccacttt tatgttttgt 1860

tttgttttgt tttttatttt gtgtgtgtgt gtaatttttt gtctgtattt ccatagttcc 1920

acagcataag ttattagaat actttgctgt taattcttga gttgcttctt gcttttagac 1980

agtgtctttc tggttggcag ctttataaac ctgtctttct ggcactagag tttccaaaca 2040

ttttctgatc tccactttta ttctctacag tgttcttgac agaagcctgg cattccctct 2100

gacatttttc tacatgttgg ggttttcatc ccaagtctta gggttgcaag ttaaatgcat 2160

tgcctcttca gacatctcat atcatgtcta ctgcttacag ttcaagaatc tttctctaaa 2220

ttactagaac gatgttcaaa gtggaataat aaataaataa ataatcaaca att 2273

<210> SEQ ID NO 15
<211> LENGTH: 2275
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 15

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcactggaga aagagttgag 60

gtggaggaat aggtaaactt cccttcctag tggtcttgaa tgtcttttac agtacatcca 120

tcaactgtta gcattttcgt aaagtcacaa aacagatatt aaactactat agttgaatct 180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca 240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta 300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg 360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg 420

agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca 480

agtatgcttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga 540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtctcaatg caaggacaag 600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc 660

aatctgcaac aaggccaaca ccagataatg cacagatgcc agtagacacc acacaagata 720

gattattggc cacaggacaa gaaaacagtg aaaatgaatg caacacctct tgtaatgcta 780

ctgaaggaaa tgttggtgag agctgtagtg gaaatgaaat ggactcctct cttattatcc 840

agaaagaaca gtaccctgag catgaagagg ggaatgttgt ttgtcaattc cctcttgatg 900

ccagaagagc aagtcaaggc acctccagtc atcatgtaga cttcctgagt gctctgacta 960

ctgccgatgt ccccatggag gaacaaccaa aggatttatc cagagaaaac atctctgagg 1020

acaagaacaa ttgctataac acttccagga atgcagctac taaagtatat agtggtgata 1080

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catctacatg cctgcaagag tcacttgggg gatgtttttc cgaaaaagac cctagggagg 1260

taccagggtt gcagtctagg taagagcagc ctatctctga tcctgtcctt cttggtaaga 1320

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tatacaagtg tgaagaatgt tctaggatgt tcaaacatgc caggagcctt tcatcccacc 1440

agagaactca cctgaataag aagagtgaat tgctttgtgt cacctgtcag aaaattttca 1500

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aacgagtctc tgaccgccga acccatgaga tcatacacat gccagaaaag cctttcaagt   1560

gcagcacatg tgaaaagtcc ttcagccaca agaccaacct gaagtctcat gagatgattc   1620

acacaggaga aatgccttat gtctgttccc tatgtagccg tcgctttcgc caatcatcca   1680

cttaccatcg tcacctgagg aattatcaca gatctgactg aagtatctaa catcctcagc   1740

agagactggt agggcttcag cctcagtatg tcatcttcaa agagagaaga atgttgcaag   1800

taaattgtac tgtcccaata atgatataac atgcttgtgg attgccactt ttatgttttg   1860

ttttgttttg ttttttattt tgtgtgtgtg tatgtaattt tttgtctgta tttccatagt   1920

tccacagcat aagttattag aatactttgc tgttaattct tgagttgctt cttgctttta   1980

gacagtgtct ttctggttgg cagctttata cacctgtctt tctggcacta gagtttccaa   2040

acattttctg atctccactt ttattttcta cagtggtcct gacagaggcc tgccattccc   2100

tctgacattt ttctacatgt tggggtttca tcccaagtct tagggttgca agttaaatgc   2160

attgcctctt cagacatctc atgtcatgtc tactgcttac agttcaagaa tatttctcta   2220

cattactaga acgacgttca aagtggaata ataaataaat aaataatcaa caatt        2275
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<210> SEQ ID NO 16
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 16

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Met Ala Ser Gln Gln Ala Pro Ala Lys Asp Leu Gln Thr Asn Asn Leu
1               5                   10                  15

Glu Phe Thr Pro Thr Asp Ser Ser Gly Val Gln Trp Ala Glu Asp Ile
            20                  25                  30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
        35                  40                  45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser
    50                  55                  60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65                  70                  75                  80

Val Leu Glu Gln Phe Leu Leu Thr Gly His Cys Lys Asp Lys Tyr Ala
                85                  90                  95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
            100                 105                 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
        115                 120                 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
    130                 135                 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Thr
145                 150                 155                 160

Pro Asp Asn Ala Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
                165                 170                 175

Ala Thr Gly Gln Glu Asn Ser Glu Asn Glu Cys Asn Thr Ser Cys Asn
            180                 185                 190

Ala Thr Glu Gly Asn Val Gly Glu Ser Cys Ser Gly Asn Glu Met Asp
        195                 200                 205

Ser Ser Leu Ile Ile Gln Lys Glu Gln Tyr Pro Glu His Glu Glu Gly
    210                 215                 220

Asn Val Val Cys Gln Phe Pro Leu Asp Ala Arg Arg Ala Ser Gln Gly
225                 230                 235                 240

Thr Ser Ser His His Val Asp Phe Leu Ser Ala Leu Thr Thr Ala Asp
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               245                 250                 255

Val Pro Met Glu Glu Gln Pro Lys Asp Leu Ser Arg Glu Asn Ile Ser
            260                 265                 270

Glu Asp Lys Asn Asn Cys Tyr Asn Thr Ser Arg Asn Ala Ala Thr Lys
        275                 280                 285

Val Tyr Ser Gly Asp Asn Ile Pro Arg Lys Lys Thr Asp Ser Leu Ser
    290                 295                 300

Ile Asn Lys Arg Ile Tyr His Pro Glu Pro Glu Val Gly Asp Ile Pro
305                 310                 315                 320

Tyr Gly Val Pro Gln Asp Ser Thr Arg Ala Ser Gln Gly Thr Ser Thr
                325                 330                 335

Cys Leu Gln Glu Ser Leu Gly Gly Cys Phe Ser Glu Lys Asp Pro Arg
            340                 345                 350

Glu Val Pro Gly Leu Gln Ser Arg
        355                 360


<210> SEQ ID NO 17
<211> LENGTH: 1774
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 17

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcactggaga aagaggtgat     60

gtggagaagt aggtaaactt ccctttcttg tggtcttgaa tgtcttttac agtacatccg    120

tcaactgtta gcattttcct aaagtcacaa aacagatact aaactgctat agttgaatct    180

ttcagaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca    240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta    300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg    360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg    420

agcagatgat ttctcaattg gtcttggagc agtttctcct cactgggcac tgcaaggaca    480

agtatgcttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga    540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtttcaatg caaggacaag    600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc    660

aatctgcaac aaggccaata ccagataatg cacagatgcc agtagacacc acacaagata    720

gattattggc cacaggcaag aaaacagtga aaatgaatgc aacacctctt gcaatgctac    780

tgaagtaaat gttggtgaaa gctgtagtgg aaatgaaaag gactcccttc ttattaccca    840

gaaagaacaa aaccatgagc atgaagaggg gaatgttgtt tgtcaattcc ctcgtggtgc    900

cagaagagca agtcaagaca cctccagtca tcatgtagac ttcccgagtg ctctgactcc    960

tgcagatgtc cccatggagg aacaaccaat ggatttatcc agagaaaaca tctctgagga   1020

caagaacaat tgctataaca cttccaggaa tgcagctact caagtatata gtggtgataa   1080

tattcccagg aacaagacag actccctttt cattaacaag agaatatatc atcctgagcc   1140

tgaggtggga gatattcctt atggagttcc tcaggattct acaagagcaa gtcaaggaac   1200

atctacatgc ctgcaagagt cacttgggga atgtttttct gaaaaagacc caagggaggt   1260

accagggttg cagtctaggc aagagcagcc tatctctgat cctgtccttg gtaagaatca   1320

tgaggcaaac ttaccatgtg aaagtcatca aaagagattc catagagatg ccaaactata   1380

caagtgtgaa gaatgttcta ggatgttcaa acatgccagg agcctttcat cccaccagag   1440

aactcacctg aataagaaga gtgaattgct ttgcatcacc tgtcagaaaa tattcaaacg   1500
```

agtttctgac cttcgaaccc atgagatcat acacatgtca gaaaagcctt tcaagtgcag 1560

cacatgtgaa aagtccttca gccacaagac caacctgaag tatcatgaga tgattcacac 1620

aggagaaatg ccttatgtct gttccctatg tagccgtcgc tttcgccaat catccactta 1680

ccatcgtcac ctgaggaatt accacagatc tgactgaagt atctaacatc ctcagcagag 1740

actggtaggg cttcagcctc agtatgtcat cttc 1774

<210> SEQ ID NO 18
<211> LENGTH: 195
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

Met Ala Ser Gln Gln Ala Pro Ala Lys Asp Leu Gln Thr Asn Asn Leu
1 5 10 15

Glu Phe Thr Pro Thr Asp Ser Ser Gly Val Gln Trp Ala Glu Asp Ile
20 25 30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
35 40 45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser
50 55 60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65 70 75 80

Val Leu Glu Gln Phe Leu Leu Thr Gly His Cys Lys Asp Lys Tyr Ala
85 90 95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
100 105 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
115 120 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
130 135 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Ile
145 150 155 160

Pro Asp Asn Ala Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
165 170 175

Ala Thr Gly Lys Lys Thr Val Lys Met Asn Ala Thr Pro Leu Ala Met
180 185 190

Leu Leu Lys
195

<210> SEQ ID NO 19
<211> LENGTH: 2275
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcaccggaga aagcagtgag 60

gtggaggaat aggtaaactt tccttcctag tggtcttgaa tgtcttttac agtacatcca 120

tcaactgtta gcattttcgt aaagtcacaa aacagatatt aaactactat agttgaatct 180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca 240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta 300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg 360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg 420

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agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca 480

agtatgcttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga 540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtttcaatg caaggacaag 600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc 660

aatctgcaac aaggccaaca ccagataatg agcagatgcc agtagacacc acacaagata 720

gattattggc cacaggacaa gaaaacagtg aaaatgaatg caacaactct tgtaatgcta 780

ctgaagcaaa tgttggtgaa agctgtagtg gaaatgaaat ggactccctt cttattatcc 840

agaaagaaca gcaccctgag catgaagagg ggaatgttgt ttgtcaattc cctcatggtg 900

ccagaagagc aagtcaaggc acccccagtc atcatgtaga cttcccgagt gctccgacta 960

ctgccgatgt ccccatggag gaacaaccaa aggatttatc cagagaaaac atctctgagg 1020

acaagaacaa ttgctataac acttccagaa atgcagctac tcaagtatat agtggtgata 1080

atattcccag gaacaagtca gactcccttt tcattaacaa gagaatatat catcctgagc 1140

ctgaggtggg agatattcct tatggagttc ctcaggattc tacaagagca agtcaaggaa 1200

catctacatg cctgcaagag tcacttgggg aatgtttttc tgaaaacgac ccaagggagg 1260

taccagggtt gcagtctagg caagagcagc ctatctctga tcctgtcctt cttggtaaga 1320

atcatgaggc aaacttacca tgtgaaagtc atcaaaagag attctgtaga gatgccaaac 1380

tatacaagtg tgaagaatgt tctaggatgt tcaaacatgc caggagcctt tcatcccacc 1440

agagaactca cctgaataag aagagtgaat tgctttgtgt cacctgtcag aaaatgttca 1500

aacgagtctc tgaccgccga acccatgaga tcatacacat gccagaaaag cctttcaagt 1560

gcagcacatg tgaaaagtcc ttcagccaca agaccaacct gaagtctcat gagatgattc 1620

acacaggaga aatgccttat gtctgttccc tatgtagccg tcgctttcgc caatcatcca 1680

cttaccatcg tcacctgagg aattaccaca gatctgactg aactatctaa catcctcagc 1740

agagactggt agggcttcag cctcagtatg tcatcttcaa agagagaaga atgttgcaag 1800

taaattgtac tgtcccaata atgatataac atgcttgtgg attgccactt ttatgttttg 1860

ttttgttttg ttwtttatkt tgtgtgtgtg tatgtaattt tttgtctgta tttccatatt 1920

tccacagcat aagttattag aatactttgc tgttaattct tgagttgctt cttgctttta 1980

gacagtgtct ttctggttgg cagctttata cacctgtctt tctggcacta gagtttccaa 2040

acatttttctg atctccactt ttattttcta cagtgttctt gacagaagcc tggcattccc 2100

tctgacattt tctacatgtt ggggttttca tcccaagtct tagggttgca agttaaatgc 2160

attgcctctt cagacatctc atgccatgtc tactgcttac agttcaagaa tatttctcta 2220

cattactaga acgacgttca aagtggaata ataaataaat aaataatcaa caatt 2275

<210> SEQ ID NO 20
<211> LENGTH: 506
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

Met Ala Ser Gln Gln Ala Pro Ala Lys Asp Leu Gln Thr Asn Asn Leu
1 5 10 15

Glu Phe Thr Pro Thr Asp Ser Ser Gly Val Gln Trp Ala Glu Asp Ile
20 25 30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
35 40 45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser

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    50                  55                  60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65                  70                  75                  80

Val Leu Glu Gln Phe Leu Leu Thr Gly His Cys Lys Asp Lys Tyr Ala
                85                  90                  95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
            100                 105                 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
        115                 120                 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
    130                 135                 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Thr
145                 150                 155                 160

Pro Asp Asn Glu Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
                165                 170                 175

Ala Thr Gly Gln Glu Asn Ser Glu Asn Glu Cys Asn Asn Ser Cys Asn
            180                 185                 190

Ala Thr Glu Ala Asn Val Gly Glu Ser Cys Ser Gly Asn Glu Met Asp
        195                 200                 205

Ser Leu Leu Ile Ile Gln Lys Glu Gln His Pro Glu His Glu Glu Gly
    210                 215                 220

Asn Val Val Cys Gln Phe Pro His Gly Ala Arg Arg Ala Ser Gln Gly
225                 230                 235                 240

Thr Pro Ser His His Val Asp Phe Pro Ser Ala Pro Thr Thr Ala Asp
                245                 250                 255

Val Pro Met Glu Glu Gln Pro Lys Asp Leu Ser Arg Glu Asn Ile Ser
            260                 265                 270

Glu Asp Lys Asn Asn Cys Tyr Asn Thr Ser Arg Asn Ala Ala Thr Gln
        275                 280                 285

Val Tyr Ser Gly Asp Asn Ile Pro Arg Asn Lys Ser Asp Ser Leu Phe
    290                 295                 300

Ile Asn Lys Arg Ile Tyr His Pro Glu Pro Glu Val Gly Asp Ile Pro
305                 310                 315                 320

Tyr Gly Val Pro Gln Asp Ser Thr Arg Ala Ser Gln Gly Thr Ser Thr
                325                 330                 335

Cys Leu Gln Glu Ser Leu Gly Glu Cys Phe Ser Glu Asn Asp Pro Arg
            340                 345                 350

Glu Val Pro Gly Leu Gln Ser Arg Gln Glu Gln Pro Ile Ser Asp Pro
        355                 360                 365

Val Leu Leu Gly Lys Asn His Glu Ala Asn Leu Pro Cys Glu Ser His
    370                 375                 380

Gln Lys Arg Phe Cys Arg Asp Ala Lys Leu Tyr Lys Cys Glu Glu Cys
385                 390                 395                 400

Ser Arg Met Phe Lys His Ala Arg Ser Leu Ser Ser His Gln Arg Thr
                405                 410                 415

His Leu Asn Lys Lys Ser Glu Leu Leu Cys Val Thr Cys Gln Lys Met
            420                 425                 430

Phe Lys Arg Val Ser Asp Arg Arg Thr His Glu Ile Ile His Met Pro
        435                 440                 445

Glu Lys Pro Phe Lys Cys Ser Thr Cys Glu Lys Ser Phe Ser His Lys
    450                 455                 460

Thr Asn Leu Lys Ser His Glu Met Ile His Thr Gly Glu Met Pro Tyr
465                 470                 475                 480
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Val Cys Ser Leu Cys Ser Arg Arg Phe Arg Gln Ser Ser Thr Tyr His
                485                 490                 495

Arg His Leu Arg Asn Tyr His Arg Ser Asp
            500                 505


<210> SEQ ID NO 21
<211> LENGTH: 2268
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcactggaca aagaggtgag     60

gtggaggagt aggtaaactt cccttcctag tggtcgtgaa tgtcttttac agtacatcca    120

tcaactgtta gcattttcat aaagtcacaa aacagatact aaactgctat agttgaatct    180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca    240

acaatttaga gtttactcca tctcatagtt ctggtgtgca gtgggtagaa gacatctcta    300

actcaccaag tgctcagcta aacttttctc caagtaacaa tggctgctgg gcaactcagg    360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg    420

agcagatgat ttctcaactg gtcttggagc agtttctcct cattgggcac tgcaaggaca    480

agtatgcttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga    540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtttcaatg caaggacaag    600

aagctctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc    660

aatctgcaac aaggccaaca ccagataatg agcagatgcc agtagacacc acacaagata    720

gattattggc cacaggacaa gaaaacagtg aaaatgaatg caacaactct tgtaatgcta    780

ctgaagcaaa tgttggtgaa agctgtagtg gaaatgaaat ggactccctt cttattatcc    840

agaaagaaca gcaccctgag catgaagagg ggaatgttgt ttttcaattc cctcttgatg    900

ccagaagagc aagtcaaggc aactccagtc atcatgtaga cttccggagt gctccgactc    960

ctgcggatgt ccccatggag gaacaaccaa aggatttatc cagagaaaac atctctgagg   1020

acaagaacaa ttgctataac acttccagga atgcagctac tcaagtatat agaagtgata   1080

atattcccag gaaaaagaca gactcccttt ccattaacaa gagaatatat cattctgagc   1140

ctgaggaggg agatattcct tatggagttc ctcaggattc tacaagagca agtcaaggaa   1200

catctacatg cttgcaagag tcacttgggg aatgtttttc tgaaaaagac cctagggagc   1260

taccagggtt ggagtctagg caagaggagc ctatctctga tcctgtcttt cttggtaagg   1320

atcatgaggc aaacttacca tgtgaaagtc atcaaaagag attccgtaga gatgccaaac   1380

tattcaagtg tgaagaatgt tctaggatgt tcaaacatgc caggagcctt tcgtcccacc   1440

agagaactca cctgaataag aagagtgaat tgctttgtgt cacctgtcag aaaatgttca   1500

aacgagtctc tgaccgccga acccatgaga tcatacacat gccagaaaag cctttcaagt   1560

gcagcacatg tgaaaagtcc ttcagccaca agaccaacct gaagtctcat gagatgattc   1620

acacaggaga aatgccttat gtctgttccc tatgtagccg tcgctttcgc caatcatcca   1680

cttaccatcg tcacctgagg aattaccaca gatctgactg aagtatctaa catcctcagc   1740

agagactggt agggcttcag cctcagtatg tcatcttcaa agagagaaga atgttgcaag   1800

taaattgtac tgtcccaata atgatataac atgcttgtgg attgccactt ttatgttttg   1860

ttttttattg tgtgtgtgtg tgtatgtaat tttttgtctg taatttccat agttccacag   1920

cataagttat tagaatactt tgctgttaat tcttgagttg cttcttgctt ttagacagtg   1980
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tctttctggt tggcagcttt atacacctgt ctttctggca ctagagtttc caaacatttt 2040

ctgatctcca cttttattct ctacagtggt cctgacagag gcctgccatt ccctctgaca 2100

ttttttaaca tgttggggtt tcatcccaag tcttagggtt gcaagttaaa tgcattgcct 2160

cttcagacat ctcatgtcat gtctactgct tacagttcaa gaatatttct ctacattact 2220

agaatgacgt tcaaagtgga ataataaata aaaaaataat caacaatt 2268

<210> SEQ ID NO 22
<211> LENGTH: 506
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 22

Met Ala Ser Gln Gln Ala Pro Ala Lys Asp Leu Gln Thr Asn Asn Leu
1 5 10 15

Glu Phe Thr Pro Ser His Ser Ser Gly Val Gln Trp Val Glu Asp Ile
20 25 30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
35 40 45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser
50 55 60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65 70 75 80

Val Leu Glu Gln Phe Leu Leu Ile Gly His Cys Lys Asp Lys Tyr Ala
85 90 95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
100 105 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
115 120 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
130 135 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Thr
145 150 155 160

Pro Asp Asn Glu Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
165 170 175

Ala Thr Gly Gln Glu Asn Ser Glu Asn Glu Cys Asn Asn Ser Cys Asn
180 185 190

Ala Thr Glu Ala Asn Val Gly Glu Ser Cys Ser Gly Asn Glu Met Asp
195 200 205

Ser Leu Leu Ile Ile Gln Lys Glu Gln His Pro Glu His Glu Glu Gly
210 215 220

Asn Val Val Phe Gln Phe Pro Leu Asp Ala Arg Arg Ala Ser Gln Gly
225 230 235 240

Asn Ser Ser His His Val Asp Phe Arg Ser Ala Pro Thr Pro Ala Asp
245 250 255

Val Pro Met Glu Glu Gln Pro Lys Asp Leu Ser Arg Glu Asn Ile Ser
260 265 270

Glu Asp Lys Asn Asn Cys Tyr Asn Thr Ser Arg Asn Ala Ala Thr Gln
275 280 285

Val Tyr Arg Ser Asp Asn Ile Pro Arg Lys Lys Thr Asp Ser Leu Ser
290 295 300

Ile Asn Lys Arg Ile Tyr His Ser Glu Pro Glu Glu Gly Asp Ile Pro
305 310 315 320

Tyr Gly Val Pro Gln Asp Ser Thr Arg Ala Ser Gln Gly Thr Ser Thr
325 330 335

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Cys Leu Gln Glu Ser Leu Gly Glu Cys Phe Ser Glu Lys Asp Pro Arg
            340                 345                 350

Glu Leu Pro Gly Leu Glu Ser Arg Gln Glu Glu Pro Ile Ser Asp Pro
        355                 360                 365

Val Phe Leu Gly Lys Asp His Glu Ala Asn Leu Pro Cys Glu Ser His
    370                 375                 380

Gln Lys Arg Phe Arg Arg Asp Ala Lys Leu Phe Lys Cys Glu Glu Cys
385                 390                 395                 400

Ser Arg Met Phe Lys His Ala Arg Ser Leu Ser Ser His Gln Arg Thr
                405                 410                 415

His Leu Asn Lys Lys Ser Glu Leu Leu Cys Val Thr Cys Gln Lys Met
            420                 425                 430

Phe Lys Arg Val Ser Asp Arg Arg Thr His Glu Ile Ile His Met Pro
        435                 440                 445

Glu Lys Pro Phe Lys Cys Ser Thr Cys Glu Lys Ser Phe Ser His Lys
    450                 455                 460

Thr Asn Leu Lys Ser His Glu Met Ile His Thr Gly Glu Met Pro Tyr
465                 470                 475                 480

Val Cys Ser Leu Cys Ser Arg Arg Phe Arg Gln Ser Ser Thr Tyr His
                485                 490                 495

Arg His Leu Arg Asn Tyr His Arg Ser Asp
            500                 505


<210> SEQ ID NO 23
<211> LENGTH: 1774
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 23

cacagtgcct ccctgggctt cttggcatca ccattgaagt tcactggaga aagaggtgag     60

gtggagaagt aggtaaactt ccctttcttg tggtcttgaa tgtcttttac agtacatccg    120

tcaactgtta gcattttcct aaagtcacaa aacagatact aaactgctat agttgaatct    180

ttcagaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca    240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta    300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg    360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg    420

agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca    480

agtatgcttt gacagagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga    540

gtctgactga tgagtgcttg aagcctcctg tcatggtcca tgtttcaatg caaggacaag    600

aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc    660

aatctgcaac aaggccaata ccagataatg agcagatgcc agtagacacc acacaagata    720

gattattggc cacaggcaag aaaacagtga aaatgaatgc aacacctctt gcaatgctac    780

tgaagtaaat gttggtgaaa gctgtagtgg aaatgaaaag gactcccttc ttattaccca    840

gaaagaacaa aaccatgagc atgaagaggg gaatgttgtt tgtcaattcc ctcgtggtgc    900

cagaagagca agtcaagaca cctccagtca tcatgtagac ttcccgagtg ctctgactcc    960

tgcagatgtc cccatggagg aacaaccaat ggatttatcc agagaaaaca tctctgagga   1020

caagaacaat tgctataaca cttccaggaa tgcagctact caagtatata atggtgataa   1080

tattcccagg aacaagacag actccctttt cattaacaag agaatatatc atcctgagcc   1140
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tgaggtggga gatattcctt atggagttcc tcaggattct acaagagcaa gtcaaggaac 1200

atctacatgc ctgcaagagt cacttgggga atgtttttct gaaaaagacc caagggaggt 1260

accagggttg cagtctaggc aagagcagcc tatctctgat cctgtccttg gtaagaatca 1320

tgaggcaaac ttaccatgtg aaagtcatca aaagagattc catagagatg ccaaactata 1380

caagtgtgaa gaatgttcta ggatgttcaa acatgccagg agcctttcat cccaccagag 1440

aactcacctg aataagaaga gtgaattgct ttgcatcacc tgtcagaaaa tattcaaacg 1500

agtttctgac cttcgaaccc atgagatcat acacatgtca gaaaagcctt tcaagtgcag 1560

cacatgtgaa aagtccttca gccacaagac caacctgaag tatcatgaga tgattcacac 1620

aggagaaatg ccttatgtct gttccctatg tagccgtcgc tttcgccaat catccactta 1680

ccatcgtcac ctgaggaatt accacagatc tgactgaagt atctaacatc ctcagcagag 1740

actggtaggg cttcagcctc agtatgtcat cttc 1774

<210> SEQ ID NO 24
<211> LENGTH: 195
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 24

Met Ala Ser Gln Gln Ala Pro Ala Lys Asp Leu Gln Thr Asn Asn Leu
1 5 10 15

Glu Phe Thr Pro Thr Asp Ser Ser Gly Val Gln Trp Ala Glu Asp Ile
20 25 30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
35 40 45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser
50 55 60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65 70 75 80

Val Leu Glu Gln Phe Leu Leu Thr Gly His Cys Lys Asp Lys Tyr Ala
85 90 95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
100 105 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
115 120 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
130 135 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Ile
145 150 155 160

Pro Asp Asn Glu Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
165 170 175

Ala Thr Gly Lys Lys Thr Val Lys Met Asn Ala Thr Pro Leu Ala Met
180 185 190

Leu Leu Lys
195

<210> SEQ ID NO 25
<211> LENGTH: 2273
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 25

cacagtgcct ccctgggctt cttggcatca cccttgaagt tcactggaga aagaggtgag 60

gtggaggaat aggtaaactt tccttcctag tggtcttgaa tgtcttttac agtacatcca 120

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tcaactgtta gcattttcgt aaagtcacaa aacagatatt aaactactat agttgaatct 180

ttcacaccat tgtcaccaca atggcttcac agcaggcacc agcaaaagac cttcagacca 240

acaatttaga gtttactcca actgatagtt ctggtgtgca gtgggcagaa gacatctcta 300

actcaccaag tgctcagcta aacttttccc caagtaacaa tggctgctgg gcaactcagg 360

agctgcaaag tctctggaag atgttcaact cctggttgca gccagaaaag cagactaagg 420

agcagatgat ttctcaactg gtcttggagc agtttctcct cactgggcac tgcaaggaca 480

agtatgcttt gactgagaag tggaaagcca gtggtagcga tatgaggaga ttcatggaga 540

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aagccctctt ttctgaaaac atgccattaa aagaagtcat caagcttttg aaacaacagc 660

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<210> SEQ ID NO 26
<211> LENGTH: 506
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 26

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            20                  25                  30

Ser Asn Ser Pro Ser Ala Gln Leu Asn Phe Ser Pro Ser Asn Asn Gly
        35                  40                  45

Cys Trp Ala Thr Gln Glu Leu Gln Ser Leu Trp Lys Met Phe Asn Ser
    50                  55                  60

Trp Leu Gln Pro Glu Lys Gln Thr Lys Glu Gln Met Ile Ser Gln Leu
65                  70                  75                  80

Val Leu Glu Gln Phe Leu Leu Thr Gly His Cys Lys Asp Lys Tyr Ala
                85                  90                  95

Leu Thr Glu Lys Trp Lys Ala Ser Gly Ser Asp Met Arg Arg Phe Met
            100                 105                 110

Glu Ser Leu Thr Asp Glu Cys Leu Lys Pro Pro Val Met Val His Val
        115                 120                 125

Ser Met Gln Gly Gln Glu Ala Leu Phe Ser Glu Asn Met Pro Leu Lys
    130                 135                 140

Glu Val Ile Lys Leu Leu Lys Gln Gln Gln Ser Ala Thr Arg Pro Thr
145                 150                 155                 160

Pro Asp Asn Glu Gln Met Pro Val Asp Thr Thr Gln Asp Arg Leu Leu
                165                 170                 175

Ala Thr Gly Gln Glu Asn Ser Glu Asn Glu Cys Asn Asn Ser Cys Asn
            180                 185                 190

Ala Thr Glu Ala Asn Val Gly Glu Ser Cys Ser Gly Asn Glu Met Asp
        195                 200                 205

Ser Leu Leu Ile Met Gln Lys Glu Gln His Pro Glu His Glu Glu Gly
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Asn Val Val Cys Gln Phe Pro His Gly Ala Arg Arg Ala Ser Gln Gly
225                 230                 235                 240

Thr Pro Ser His His Val Asp Phe Pro Ser Ala Pro Thr Thr Ala Asp
                245                 250                 255

Val Pro Met Glu Glu Gln Pro Lys Asp Leu Ser Arg Glu Asn Ile Ser
            260                 265                 270

Glu Asp Lys Asn Asn Cys Tyr Asn Thr Ser Arg Asn Ala Ala Thr Gln
        275                 280                 285

Val Tyr Ser Gly Asp Asn Ile Pro Arg Asn Lys Ser Asp Ser Leu Phe
    290                 295                 300

Ile Asn Lys Arg Ile Tyr His Pro Glu Pro Glu Val Gly Asp Ile Pro
305                 310                 315                 320

Tyr Gly Val Pro Gln Asp Ser Thr Arg Ala Ser Gln Gly Thr Ser Thr
                325                 330                 335

Cys Leu Gln Glu Ser Leu Gly Glu Cys Phe Ser Glu Lys Asp Pro Arg
            340                 345                 350

Glu Val Pro Gly Leu Gln Ser Arg Gln Glu Gln Leu Ile Ser Asp Pro
        355                 360                 365

Val Leu Leu Gly Lys Asn His Glu Ala Asn Leu Pro Cys Glu Ser His
    370                 375                 380

Gln Lys Arg Phe Cys Arg Asp Ala Lys Leu Tyr Lys Cys Glu Glu Cys
385                 390                 395                 400

Ser Arg Met Phe Lys His Ala Arg Ser Leu Ser Ser His Gln Arg Thr
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His Leu Asn Lys Lys Ser Glu Leu Leu Cys Val Thr Cys Gln Lys Met
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Phe Lys Arg Val Ser Asp Arg Arg Thr His Glu Ile Ile His Met Pro
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Glu Lys Pro Phe Lys Cys Ser Thr Cys Glu Lys Ser Phe Ser His Lys
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Thr Asn Leu Lys Ser His Glu Met Ile His Thr Gly Glu Met Pro Tyr
465                 470                 475                 480

Val Cys Ser Leu Cys Ser Arg Arg Phe Arg Gln Ser Ser Thr Tyr His
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Arg His Leu Arg Asn Tyr His Arg Ser Asp
            500                 505


<210> SEQ ID NO 27
<211> LENGTH: 1524
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 27

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cactcaccaa gtgttcagtt caatgttttc ccaaataaca atggctccct ggcaaagcag    180

gagctgcaaa cactctggga gatgtttacc tcctggttgc agccagaaaa gcagactaag    240

gagcagatga tttctcaact ggtcttggag cagtttctca tcactgggca ctgcaaggac    300

aagtatgctt tgacagagaa gtggaaagcc agtggcagaa acatggagag attcatggag    360

agtctgactg atgagtgctt gaagcctcct gtcatgatcc atgttgccat gcatgggcag    420

gaagcccttt tttctgagaa catgcccttа aaagaagtca tcacactttt ggaacaacag    480

aaagtagcaa caactccaac tcaagagaat gcaagggcac tcttggagat ccccaaagat    540

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gagggagatt ctgttttcat tatccagaga gagcagtatt ctgaacctga tgtggaaagt    960

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tccacctttg agggttacca agagagactc cagagagatc ccaaaccgta caaatgtgag   1200

gaatgtccca gaaccttcaa atatccctgc aacctctcca tccaccagaa aacacacagg   1260

aaggagaggc catttttctg taaggagtgc cagataggct ttaccaaaa gtcagaactt   1320

cacgatcatg aggtcataca caaggcagag aagcctttcg catgcagtac gtgtggaagg   1380

gccttcagat acaagaccaa cctgcaggct catgagagaa ttcacacagg agagaagcct   1440

tattcttgct ccctgtgtaa tagtagcttc cgccagtcat ccacattcca ccgtcacttg   1500

aggaagttcc acaaatcaga atga                                          1524


<210> SEQ ID NO 28
<211> LENGTH: 6017
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<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Zscan4c promoter-Emerald plasmid

<400> SEQUENCE: 28

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ttcctctcta aaagtaatgt ttttgttaag acctattaat ttgttataaa ttttgttggg 180

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aatctgtggt tgtccacaaa gtacagagcc atacatccac ccaatgatgc tatttgaata 420

ttgtcccgaa atacaactgg tcaaaaaaaa aaaaaaaaaa aagcaacttg ctatgattgg 480

tcattggagg gagaaaggtt ggatttgagg attaagtgaa gagattgctg gtagaggaag 540

agaaagaaga aagaagactt aagtggagga ggctgtcatg ggaagtgatg aaatataaat 600

tcttggaaca gagaaacagc aagtataagg gacttgatcc ttggggaata agttagaata 660

gctgtaaatc tgccttattt aggcttgagt ttataaataa aatagctaga ttgtgtttct 720

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agcatggctt ctaccttcct agtagtagcg gttccagggc aaccttacta cttctatcat 840

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<210> SEQ ID NO 29
<211> LENGTH: 2230
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

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gcccctataa aagggagcag ccttaggagg cacatcagat aaacccagtg tggaaagcta 540

gtcacacatc agctcagtgt tcggcccggg attacccagt caaccaagga gcttgcagtt 600

ttaaagaatc caccaactgt tgaaacaaat ccctagagac acaaggcaag agactgaatc 660

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ccacctgcct tagtccacgt ccacatgcag ggacaggaag ctctcttttc tgaggatatg   1140

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attactaatc aaggcaatca aatagtttcc ctaatcatca tccaggaaga gaacggtcct   1380

aggcctgaag agggaggtgt ttcttctgac aacccataca actcaaaaag agcagagcta   1440

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caccagagca atgagggaaa ttccacatgt gaggtacatc agaaaggatc ccatggagtc   1620

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gctcaccaga gaagacacag gaatgagagg ccatttgttt gtcccgagtg tcaaaaaggc   1740

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tatattccta tagtatttat ctacttagga tataagatat aatctcctga ttatgctttc   2100

aatttattgt cttgcttcat taaaatgtaa ggctaaggag agcatggaat ttgtcagttt   2160

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tctgttgaat                                                          2230


<210> SEQ ID NO 30
<211> LENGTH: 433
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

Met Ala Leu Asp Leu Arg Thr Ile Phe Gln Cys Glu Pro Ser Glu Asn
1               5                   10                  15

Asn Leu Gly Ser Glu Asn Ser Ala Phe Gln Gln Ser Gln Gly Pro Ala
            20                  25                  30

Val Gln Arg Glu Glu Gly Ile Ser Glu Phe Ser Arg Met Val Leu Asn
        35                  40                  45

Ser Phe Gln Asp Ser Asn Asn Ser Tyr Ala Arg Gln Glu Leu Gln Arg
    50                  55                  60

Leu Tyr Arg Ile Phe His Ser Trp Leu Gln Pro Glu Lys His Ser Lys
65                  70                  75                  80

Asp Glu Ile Ile Ser Leu Leu Val Leu Glu Gln Phe Met Ile Gly Gly
                85                  90                  95

His Cys Asn Asp Lys Ala Ser Val Lys Glu Lys Trp Lys Ser Ser Gly
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            100                 105                 110

Lys Asn Leu Glu Arg Phe Ile Glu Asp Leu Thr Asp Asp Ser Ile Asn
        115                 120                 125

Pro Pro Ala Leu Val His Val His Met Gln Gly Gln Glu Ala Leu Phe
    130                 135                 140

Ser Glu Asp Met Pro Leu Arg Asp Val Ile Val His Leu Thr Lys Gln
145                 150                 155                 160

Val Asn Ala Gln Thr Thr Arg Glu Ala Asn Met Gly Thr Pro Ser Gln
                165                 170                 175

Thr Ser Gln Asp Thr Ser Leu Glu Thr Gly Gln Gly Tyr Glu Asp Glu
            180                 185                 190

Gln Asp Gly Trp Asn Ser Ser Ser Lys Thr Thr Arg Val Asn Glu Asn
        195                 200                 205

Ile Thr Asn Gln Gly Asn Gln Ile Val Ser Leu Ile Ile Ile Gln Glu
    210                 215                 220

Glu Asn Gly Pro Arg Pro Glu Glu Gly Gly Val Ser Ser Asp Asn Pro
225                 230                 235                 240

Tyr Asn Ser Lys Arg Ala Glu Leu Val Thr Ala Arg Ser Gln Glu Gly
                245                 250                 255

Ser Ile Asn Gly Ile Thr Phe Gln Gly Val Pro Met Val Met Gly Ala
            260                 265                 270

Gly Cys Ile Ser Gln Pro Glu Gln Ser Ser Pro Glu Ser Ala Leu Thr
        275                 280                 285

His Gln Ser Asn Glu Gly Asn Ser Thr Cys Glu Val His Gln Lys Gly
    290                 295                 300

Ser His Gly Val Gln Lys Ser Tyr Lys Cys Glu Glu Cys Pro Lys Val
305                 310                 315                 320

Phe Lys Tyr Leu Cys His Leu Leu Ala His Gln Arg Arg His Arg Asn
                325                 330                 335

Glu Arg Pro Phe Val Cys Pro Glu Cys Gln Lys Gly Phe Phe Gln Ile
            340                 345                 350

Ser Asp Leu Arg Val His Gln Ile Ile His Thr Gly Lys Lys Pro Phe
        355                 360                 365

Thr Cys Ser Met Cys Lys Lys Ser Phe Ser His Lys Thr Asn Leu Arg
    370                 375                 380

Ser His Glu Arg Ile His Thr Gly Glu Lys Pro Tyr Thr Cys Pro Phe
385                 390                 395                 400

Cys Lys Thr Ser Tyr Arg Gln Ser Ser Thr Tyr His Arg His Met Arg
                405                 410                 415

Thr His Glu Lys Ile Thr Leu Pro Ser Val Pro Ser Thr Pro Glu Ala
            420                 425                 430

Ser


<210> SEQ ID NO 31
<211> LENGTH: 4996
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 31

ccaaagaggt tctatgggaa cccctaaaca actcaggata ttgtcaaaac tacatttcct     60

tctctctcca gctcttatca taactaaatc cactgcccag gggccaatat ctgattctct    120

agaatataaa gacaaaggag tactataagg tcagtcagct cagtaggctg aattattggt    180

actcactcag ttgtgggtgt catctgtgga cccaccacac ccaggtaaag aaagcaactc    240
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atccagaaca ataaagactt ggtcatcaaa aatccatcta gccaggcttg gtggcacact 300

cctttaatct accccccttt tttagattta gtgtttctct gtgtagccct ggctgatctt 360

caattcactt tgtagagtag gctgtactag aactgagagc tccacctgcc tctgctttac 420

tttcattaca tggttatcag tctgtgcatt gaagacctta ggaggggtat tttacttaag 480

attttggtaa taaaaacaaa tattgtctga tcattgtggt acataccttt aatcccagga 540

ctttggaggc agaggcaagt ggatttaagc ccagtttaaa atctgattcc aggacagcag 600

gagctacata aaagagagcc tgtctccaaa aacaaacaaa caaacaaaca aacaaacagt 660

ccccaaccaa aaaaacaaaa caaaacaaaa cacaacaacc aaccaaccaa ccatcatatg 720

aaaccattta aagataaata aaaccaaaaa tttacaccca ctttttataa aagtagtata 780

attcttccta ggttttgtgt ttcatactca aataatattg ccatccagtg gcatttaatg 840

tgaaaatttc tttcaaaggc ctgtgtgcta agtaaaactt agcccagtgt gtgctagtgt 900

tcatttaaac aacacccccc ctctctgaac acaaacaaat atatgttctc tgcacctcat 960

ggaactttct ctaaaactga ccacattctt ggacataaag taagtctcaa cagatagaag 1020

aaatttgaaa taactcagtg tatcctgtga ggccaccaca gaataaagct tgatatcaac 1080

aacaaagaaa caacagaaag ctcacaaaac acatggaaac tatacaattt actactgcat 1140

gaagaccaaa gtaaagaaat taaagacccc atagaattga ctagaaatgc atatacacca 1200

tacccaaaat catgggacac gagaaagggg gaagttcttt tatttttatt ttcctgagac 1260

agggtttctc tgtatagccc tggctttcct ggaactcact ttgtagacca ggctggcctt 1320

gaactcagaa atctgcctgc ctcccaagtg ctgggattaa aggtgtgtgc caccactgcc 1380

ctgctaaagg ggaatgttct aaaggacaag gtcacaggac caaatgccta caaaaacaaa 1440

caaacaacta aagagaagcc aaggggtgga gtctcacacc tttaatccca tcccttgaga 1500

ggaaggtggg tctctgagtt caagttcagc ctagtctgca gatccaattc caggactgcc 1560

aaggctacaa agagaaaccg tgtttctgga aaagagaagc agacctagag aaatcttgta 1620

ctagcaactt aacagcacac ctaaaagctc taggacaacc acaggaagaa ggagtacacg 1680

gcaagaaata aactgaggac tgaaatcaat aaaatagaaa caaagggaac ccttcatcag 1740

ttctttgaga aaatcagcgg gattgcaaac ccttattcaa attaactgac agaccccaga 1800

gagagagaac aggcagatga acaaaatcaa aatgaaagag ggtggtgagg tggggaagtc 1860

tctaagaagt gccagagatc tgggatgggg aaggctccca ggagccaatg caggatcaag 1920

ccccatttca cttcctggtg ccccttaat agttgaataa cacattttat attttttctt 1980

ttctaaattt gctatgcctg ttttaagcgc tcgttgtgag tcttaaccag agggcaaaat 2040

ctatgctggg tatttttgag actccctttt caatgcaact aatctgagtc ttattcaact 2100

gaatctcaag cagactctta agactagggc aaaaggcagt cacattcctt caccaaatat 2160

cccaagagca gcctctagtc cacatactga catccttctc ccacagttca aatcaccctc 2220

agcatcaatg tcttccatct tcctactaga atggttcact aagcctaact taaagcactt 2280

cactactttc tacatccaaa gccagcaagt caacattccc caacccaaaa catgataaag 2340

cctatccagt aacaccccag tccccagtac caacttctgc attagttagg gctctccaga 2400

gtcagagaaa tcatcggatg tctctatata tgaaggggat ttgttgtctg cagtgcatct 2460

aacccagaaa tgggcagctg tgaatgggaa accccacttc acgagaaaaa tttattttca 2520

atgatttaaa aaaagaagtg cctaggaaat caacaggata ttcctttgag tatgtctagg 2580

agggcctttc agagaacaag agcgaagcgc catgctgtgg gcatccatcc aatagattgg 2640

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gcacataggg ataaaagaaa ggcagtgagt gagtgcaggt agtctgcctc tctgctttat 2700

ggccactgag gtgaagacct tagctctgac acctagacag agacttagct tgtatcaggg 2760

ataagctttc taactgatca cccagtacaa agtggtcagc ccttctgatg gctatgcttc 2820

gttgtaattt cactacatct ggaattaagt ataactcaag aggctggaaa ttttgttgtg 2880

aggatttttc cccctcctga ctgaatcatt tgaggcagag aaaactaccg agaccctgac 2940

attttgacca tcgagaatct acctaaaatc ctagccaaag ctctggtggc agcctctata 3000

aaggacctgt aagaagggaa gggagaggaa aacaatggaa ttttacttta actaaaatat 3060

atattaaaag cagatcatcc aggtgcaaag caagcaaaaa cctgattgag atgtggaagg 3120

ttccttgtaa tttcacagcc acaacattaa cacacgactc tgtctggtta acgtgaacta 3180

gcctggtggg gagctaggca tctttgaact ctaatgtcac tgtacacagc caaaagtaaa 3240

tagagggaga tttgtgcatt ttttcccttt tagaacagaa agtcgagtca gtaagcaggg 3300

tagatttgga agaagtataa gttgagatca atatgagcaa aagaagttgt aatagaatcc 3360

tccaaagatc taaaaagata tttatgttga tatttgctgg aatcagaatt aagggtgcca 3420

tcatttgtta agctattaaa accaaaggat aagcatattg ctcaatatgt agttatagtt 3480

attgttgcaa aattactaat ttttttcttt agaaaagctc tcatgctggg cgtggtggca 3540

caagccttta atcccagcac gtgggaggca gaggtaggca gatttctgag tatgaggcca 3600

gcctggtcta caaagtgagt tccaggacag cctatacaga gaaaccctgt ctcgaaaaac 3660

aaacaaacaa aaaaaaaaaa aaaaaaaaga aaaaaaagaa aagctctcat tgcatattct 3720

aggcaggcct tgaactaaaa aaatcctcct agttcagcat tctaattcct tggattctgg 3780

gtaaaggttt gttaccacac ccagctaaac agtgatttgg gacatccctt gggggagatt 3840

tgcttgtgga gaatggccaa ggtgttagtt caatctctca tccatttaga ataatcccac 3900

ttaaggaagc tcatctattg gaagcattag taaaagggag gaagtgggtg tggtttttag 3960

agactctaag tacatccctg gggcccacca ggttcattct tctccagacc agaggtagag 4020

tgtttctaac cttttgctcc agacactgct agatctatca cctcactctc tgaggatctg 4080

atctcagagc tgagcgagta tcgcattgct accaaccatt gctaagcagg gacgaggata 4140

attgcttggg taagtgcaca gtttacaaga gaaaatttct tttttgttcc tattttaaat 4200

acaaacaggg gtttgcttag aagttgtatt ttgctattta gcaaaacctg attcagtttg 4260

tatttgcatt ttttttcttg ggatataatg tgggttaagg ttatagataa ttttaaattt 4320

attatgcaca tgttagttga tctgatgtat tataatgaga gatagtttca agatctcctc 4380

ctcctccttc tcttccttct tcatttttca agacagggtc tctctgtgta gccctagttt 4440

tcctggaact ttctttgtag atcaggctgg ccttgaactc agaaatctcc tgcctctgcc 4500

tccctctgcc tccctctgtc tcccaagtgc tccgattaaa ggcgtagcca ccactgcctg 4560

gctcaagata ctttttttat attctgtgct ttgtctaaat tctaaaatat ttcaagaaca 4620

ttctatgctt aacaaatgct ctgagtggtt ttaagaaata tcagaattta aagcttgagg 4680

tagggtgcat tttcttggat aggaaggtgc tgtttcacta acgtgcctgc agtgaaaggc 4740

cagactggag gagaagggct tggatcactc ctcaatgaat gtctctggcc tcaaagaatg 4800

taccagtttg ggctgaagtc tccaggagga atgtagatgg taggatcacc tcaggcaata 4860

tgcctgtcag ggaaagttct tggtcataaa aaaaaaaaag cctatattgc cataatcaca 4920

agttgaatca aactttgtct agtttcttgt tcctctctgg cccaataata acactgcttt 4980

ttttcccctc agaaaa 4996

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<210> SEQ ID NO 32
<211> LENGTH: 1341
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1341)

<400> SEQUENCE: 32

```
atg gaa tca gac aat tta caa gac cct cag gag gaa aca ctc acc tgc       48
Met Glu Ser Asp Asn Leu Gln Asp Pro Gln Glu Glu Thr Leu Thr Cys
1               5                  10                  15

tcc atc tgc cag agt atc ttt atg aat cca gtt tat tta agg tgt ggc       96
Ser Ile Cys Gln Ser Ile Phe Met Asn Pro Val Tyr Leu Arg Cys Gly
            20                  25                  30

cat aag ttc tgc gag gca tgt ctc tta ctt tct caa gaa gac atc aaa      144
His Lys Phe Cys Glu Ala Cys Leu Leu Leu Ser Gln Glu Asp Ile Lys
        35                  40                  45

ttt cct gcc tac tgc ccc atg tgt atg caa cca ttt aac cag gaa tat      192
Phe Pro Ala Tyr Cys Pro Met Cys Met Gln Pro Phe Asn Gln Glu Tyr
    50                  55                  60

ata aat gac att tct ctg aag aag cag gtg tcc att gtc aga aag aaa      240
Ile Asn Asp Ile Ser Leu Lys Lys Gln Val Ser Ile Val Arg Lys Lys
65                  70                  75                  80

agg ctc atg aaa tat ttg aat tct aag gag cac aag tgt gtg acc cac      288
Arg Leu Met Lys Tyr Leu Asn Ser Lys Glu His Lys Cys Val Thr His
                85                  90                  95

aag gca aaa aag atg atc ttc tgt gat aag agc aag atc ctc ctc tgt      336
Lys Ala Lys Lys Met Ile Phe Cys Asp Lys Ser Lys Ile Leu Leu Cys
            100                 105                 110

cac ctg tgt tct gac tcc cag gag cac agt ggt cac aca cac tgt tcc      384
His Leu Cys Ser Asp Ser Gln Glu His Ser Gly His Thr His Cys Ser
        115                 120                 125

att gat gta gct gtt cag gag aaa atg gag gaa ctt cta aag cac atg      432
Ile Asp Val Ala Val Gln Glu Lys Met Glu Glu Leu Leu Lys His Met
    130                 135                 140

gac tca tta tgg cgg agg ctc aaa atc cag cag aat tat gta gaa ata      480
Asp Ser Leu Trp Arg Arg Leu Lys Ile Gln Gln Asn Tyr Val Glu Ile
145                 150                 155                 160

gag agg aga acg acc ttg tgg tgg ttg aag tcc gtg aag cta cgg gag      528
Glu Arg Arg Thr Thr Leu Trp Trp Leu Lys Ser Val Lys Leu Arg Glu
                165                 170                 175

gaa gtg atc aag aga gtg twt gga aaa caa tgt cca ccc ctc tgt gaa      576
Glu Val Ile Lys Arg Val Xaa Gly Lys Gln Cys Pro Pro Leu Cys Glu
            180                 185                 190

gaa agg gat caa cac ata gag tgt ttg aga cat caa agc aac act act      624
Glu Arg Asp Gln His Ile Glu Cys Leu Arg His Gln Ser Asn Thr Thr
        195                 200                 205

tta gag gag ctc agg aaa agt gaa gct acg ata gtc cac gag aga aat      672
Leu Glu Glu Leu Arg Lys Ser Glu Ala Thr Ile Val His Glu Arg Asn
    210                 215                 220

caa cta ata gag gtt tat cgg gag ctg atg aca atg tcc cag agg cca      720
Gln Leu Ile Glu Val Tyr Arg Glu Leu Met Thr Met Ser Gln Arg Pro
225                 230                 235                 240

tac cag gag ctg ctg gtg cag gac ttg gat gac ttg ttc aga agg agt      768
Tyr Gln Glu Leu Leu Val Gln Asp Leu Asp Asp Leu Phe Arg Arg Ser
                245                 250                 255

aag cta gcg gca aag ctg gac atg cca cag ggt atg ata cca aga ctc      816
Lys Leu Ala Ala Lys Leu Asp Met Pro Gln Gly Met Ile Pro Arg Leu
            260                 265                 270

cat gcc cat tcc att cct ggg ctg act gca agg ctc aac tcc ttc cga      864
```

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```
His Ala His Ser Ile Pro Gly Leu Thr Ala Arg Leu Asn Ser Phe Arg
        275                 280                 285

gtg aag att tcc ttt aaa cat tca atc atg ttc ggc tac acc tca gtc      912
Val Lys Ile Ser Phe Lys His Ser Ile Met Phe Gly Tyr Thr Ser Val
    290                 295                 300

aga cct ttt gat atc aga ctt ctc cat gaa agc aca tct ctg gat tca      960
Arg Pro Phe Asp Ile Arg Leu Leu His Glu Ser Thr Ser Leu Asp Ser
305                 310                 315                 320

gct gaa acc cat cgt gtt tcc tgg gga aaa aag agc ttc tcc agg gga     1008
Ala Glu Thr His Arg Val Ser Trp Gly Lys Lys Ser Phe Ser Arg Gly
                325                 330                 335

aaa tac tac tgg gag gtg gat ttg aag gac cat gag cag tgg act gta     1056
Lys Tyr Tyr Trp Glu Val Asp Leu Lys Asp His Glu Gln Trp Thr Val
            340                 345                 350

gga gtc cgt aag gat ccc tgg tta agg ggg aga agc tat gcg gcg aca     1104
Gly Val Arg Lys Asp Pro Trp Leu Arg Gly Arg Ser Tyr Ala Ala Thr
        355                 360                 365

ccc aca gat cta ttt ctt ctt gag tgt ttg aga aag gaa gat cat tac     1152
Pro Thr Asp Leu Phe Leu Leu Glu Cys Leu Arg Lys Glu Asp His Tyr
    370                 375                 380

att ctc atc acc cgc ata gga ggt gaa cac tat ata gag aag cca gtt     1200
Ile Leu Ile Thr Arg Ile Gly Gly Glu His Tyr Ile Glu Lys Pro Val
385                 390                 395                 400

ggc caa gtt ggc gtg ttc ctt gat tgt gag ggt gga tat gta agt ttc     1248
Gly Gln Val Gly Val Phe Leu Asp Cys Glu Gly Gly Tyr Val Ser Phe
                405                 410                 415

gtg gat gta gcc aag agt tcc ctc ata ctc agc tac tct cct gga act     1296
Val Asp Val Ala Lys Ser Ser Leu Ile Leu Ser Tyr Ser Pro Gly Thr
            420                 425                 430

ttc cat tgt gct gtc agg cct ttc ttc tct gct gtc tac aca taa         1341
Phe His Cys Ala Val Arg Pro Phe Phe Ser Ala Val Tyr Thr
        435                 440                 445


<210> SEQ ID NO 33
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (183)..(183)
<223> OTHER INFORMATION: The 'Xaa' at location 183 stands for Tyr, or
      Phe.

<400> SEQUENCE: 33

Met Glu Ser Asp Asn Leu Gln Asp Pro Gln Glu Glu Thr Leu Thr Cys
1               5                   10                  15

Ser Ile Cys Gln Ser Ile Phe Met Asn Pro Val Tyr Leu Arg Cys Gly
            20                  25                  30

His Lys Phe Cys Glu Ala Cys Leu Leu Leu Ser Gln Glu Asp Ile Lys
        35                  40                  45

Phe Pro Ala Tyr Cys Pro Met Cys Met Gln Pro Phe Asn Gln Glu Tyr
    50                  55                  60

Ile Asn Asp Ile Ser Leu Lys Lys Gln Val Ser Ile Val Arg Lys Lys
65                  70                  75                  80

Arg Leu Met Lys Tyr Leu Asn Ser Lys Glu His Lys Cys Val Thr His
                85                  90                  95

Lys Ala Lys Lys Met Ile Phe Cys Asp Lys Ser Lys Ile Leu Leu Cys
            100                 105                 110

His Leu Cys Ser Asp Ser Gln Glu His Ser Gly His Thr His Cys Ser
        115                 120                 125
```

-continued

```
Ile Asp Val Ala Val Gln Glu Lys Met Glu Glu Leu Leu Lys His Met
    130                 135                 140

Asp Ser Leu Trp Arg Arg Leu Lys Ile Gln Gln Asn Tyr Val Glu Ile
145                 150                 155                 160

Glu Arg Arg Thr Thr Leu Trp Trp Leu Lys Ser Val Lys Leu Arg Glu
                165                 170                 175

Glu Val Ile Lys Arg Val Xaa Gly Lys Gln Cys Pro Pro Leu Cys Glu
            180                 185                 190

Glu Arg Asp Gln His Ile Glu Cys Leu Arg His Gln Ser Asn Thr Thr
        195                 200                 205

Leu Glu Glu Leu Arg Lys Ser Glu Ala Thr Ile Val His Glu Arg Asn
    210                 215                 220

Gln Leu Ile Glu Val Tyr Arg Glu Leu Met Thr Met Ser Gln Arg Pro
225                 230                 235                 240

Tyr Gln Glu Leu Leu Val Gln Asp Leu Asp Asp Leu Phe Arg Arg Ser
                245                 250                 255

Lys Leu Ala Ala Lys Leu Asp Met Pro Gln Gly Met Ile Pro Arg Leu
            260                 265                 270

His Ala His Ser Ile Pro Gly Leu Thr Ala Arg Leu Asn Ser Phe Arg
        275                 280                 285

Val Lys Ile Ser Phe Lys His Ser Ile Met Phe Gly Tyr Thr Ser Val
    290                 295                 300

Arg Pro Phe Asp Ile Arg Leu Leu His Glu Ser Thr Ser Leu Asp Ser
305                 310                 315                 320

Ala Glu Thr His Arg Val Ser Trp Gly Lys Lys Ser Phe Ser Arg Gly
                325                 330                 335

Lys Tyr Tyr Trp Glu Val Asp Leu Lys Asp His Glu Gln Trp Thr Val
            340                 345                 350

Gly Val Arg Lys Asp Pro Trp Leu Arg Gly Arg Ser Tyr Ala Ala Thr
        355                 360                 365

Pro Thr Asp Leu Phe Leu Leu Glu Cys Leu Arg Lys Glu Asp His Tyr
    370                 375                 380

Ile Leu Ile Thr Arg Ile Gly Gly Glu His Tyr Ile Glu Lys Pro Val
385                 390                 395                 400

Gly Gln Val Gly Val Phe Leu Asp Cys Glu Gly Gly Tyr Val Ser Phe
                405                 410                 415

Val Asp Val Ala Lys Ser Ser Leu Ile Leu Ser Tyr Ser Pro Gly Thr
            420                 425                 430

Phe His Cys Ala Val Arg Pro Phe Phe Ser Ala Val Tyr Thr
        435                 440                 445


<210> SEQ ID NO 34
<211> LENGTH: 886
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (189)..(680)

<400> SEQUENCE: 34

gcaagtctat cagtttgagg gtactagagc aagctggtct gtgattccat cttctactga     60

taaccaattg agacatccag cctcagtgag tgagaacttc tggattcttg gacttttctt    120

caaattcagc tggtgtggaa taagctcgac tgcaacctaa agtcaaggac tttggtgaag    180

ccaaggca atg aag cgg ttc tgt ccc tgt ctt gtc caa gat aca tca cat     230
         Met Lys Arg Phe Cys Pro Cys Leu Val Gln Asp Thr Ser His
         1               5                   10
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tcc gaa gag cat gca ctg cag act tca caa gaa ttg cca gcc ctg aga      278
Ser Glu Glu His Ala Leu Gln Thr Ser Gln Glu Leu Pro Ala Leu Arg
15                  20                  25                  30

cca cga tat tcc agg tct gag cca cag tgt ttc tgt gga gag cca aac      326
Pro Arg Tyr Ser Arg Ser Glu Pro Gln Cys Phe Cys Gly Glu Pro Asn
                35                  40                  45

cac tgc cat gag gat gac tgg att gtt gat tgg gaa cca tac tac ctt      374
His Cys His Glu Asp Asp Trp Ile Val Asp Trp Glu Pro Tyr Tyr Leu
            50                  55                  60

ccc tgt gta ctt gaa agc tgg gac tgc ttg aga tac cac tcc gga ttg      422
Pro Cys Val Leu Glu Ser Trp Asp Cys Leu Arg Tyr His Ser Gly Leu
        65                  70                  75

aat tgt gcc atg aag aag ggc aca gag gtc ttc cag att gag agt cag      470
Asn Cys Ala Met Lys Lys Gly Thr Glu Val Phe Gln Ile Glu Ser Gln
    80                  85                  90

agg ggg cca caa gtg ttc cca gga gat atg gac aat gac aaa gat aca      518
Arg Gly Pro Gln Val Phe Pro Gly Asp Met Asp Asn Asp Lys Asp Thr
95                  100                 105                 110

gag gag cca gac caa ccc ttg cca agc ttg ctc agg gag aaa ggg ctg      566
Glu Glu Pro Asp Gln Pro Leu Pro Ser Leu Leu Arg Glu Lys Gly Leu
                115                 120                 125

gaa ctt gag acc tgt gat ggt gga gac tgc cct gac cag gat ccc gct      614
Glu Leu Glu Thr Cys Asp Gly Gly Asp Cys Pro Asp Gln Asp Pro Ala
            130                 135                 140

tct gac agt ccc aag cac cta ggc tgc tgc tta tgg ctt caa agg gct      662
Ser Asp Ser Pro Lys His Leu Gly Cys Cys Leu Trp Leu Gln Arg Ala
        145                 150                 155

ttt ggc cag aag aag tga gaaagccacc cagaactctg tgtggagccc             710
Phe Gly Gln Lys Lys
    160

aggagccctg atgcctgcta agacttgcaa tgaggggatc ctcggtcagc tcctgctatt    770

acagagagac acacccctgc ctctctcaca tccaaaggca attgtgtctt cagccatctg    830

gatgttgttt gtttgtttgt ttgttacagc tttcttaata aaagtgttaa aaagct        886


<210> SEQ ID NO 35
<211> LENGTH: 163
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 35

Met Lys Arg Phe Cys Pro Cys Leu Val Gln Asp Thr Ser His Ser Glu
1               5                   10                  15

Glu His Ala Leu Gln Thr Ser Gln Glu Leu Pro Ala Leu Arg Pro Arg
            20                  25                  30

Tyr Ser Arg Ser Glu Pro Gln Cys Phe Cys Gly Glu Pro Asn His Cys
        35                  40                  45

His Glu Asp Asp Trp Ile Val Asp Trp Glu Pro Tyr Tyr Leu Pro Cys
    50                  55                  60

Val Leu Glu Ser Trp Asp Cys Leu Arg Tyr His Ser Gly Leu Asn Cys
65                  70                  75                  80

Ala Met Lys Lys Gly Thr Glu Val Phe Gln Ile Glu Ser Gln Arg Gly
                85                  90                  95

Pro Gln Val Phe Pro Gly Asp Met Asp Asn Asp Lys Asp Thr Glu Glu
            100                 105                 110

Pro Asp Gln Pro Leu Pro Ser Leu Leu Arg Glu Lys Gly Leu Glu Leu
        115                 120                 125

Glu Thr Cys Asp Gly Gly Asp Cys Pro Asp Gln Asp Pro Ala Ser Asp
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    130                 135                 140

Ser Pro Lys His Leu Gly Cys Cys Leu Trp Leu Gln Arg Ala Phe Gly
145                 150                 155                 160

Gln Lys Lys


<210> SEQ ID NO 36
<211> LENGTH: 1625
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (102)..(1547)

<400> SEQUENCE: 36

acactcagag acctgcagcc tgataactgc ctggtgcagc tgggacttgg agacctatct     60

gcagtgctca actggagcct tctgactgga gactgaagag g atg agt gtt cag act    116
                                               Met Ser Val Gln Thr
                                               1               5

ctg tcc act ctc cag aat ctg aca ttg aag gct ctg ctg aga gat gag      164
Leu Ser Thr Leu Gln Asn Leu Thr Leu Lys Ala Leu Leu Arg Asp Glu
                10                  15                  20

gct ttg gcc ttg tcc tgt ctg gag gag gtg cct ttt ctg ctc ttc cca      212
Ala Leu Ala Leu Ser Cys Leu Glu Glu Val Pro Phe Leu Leu Phe Pro
            25                  30                  35

gca ctg ttc cag agg gcc ttt gct ggc aga ctt aag aag ctc atg aag      260
Ala Leu Phe Gln Arg Ala Phe Ala Gly Arg Leu Lys Lys Leu Met Lys
        40                  45                  50

gca atc atg gca gcc tgg act ttt ccc tgt ctc cct gtg ggg gct ttg      308
Ala Ile Met Ala Ala Trp Thr Phe Pro Cys Leu Pro Val Gly Ala Leu
    55                  60                  65

atg aag tca cct aac ctg gag acc ttg cag gct gtg cta gat gga ata      356
Met Lys Ser Pro Asn Leu Glu Thr Leu Gln Ala Val Leu Asp Gly Ile
70                  75                  80                  85

gac atg caa ctg aca aga gaa tct cac ccc agg gga aaa ctt cag gtt      404
Asp Met Gln Leu Thr Arg Glu Ser His Pro Arg Gly Lys Leu Gln Val
                90                  95                  100

ctg gac ctg agg aat gtg cac cat gcc ttc tgg gac ata tgg gct ggt      452
Leu Asp Leu Arg Asn Val His His Ala Phe Trp Asp Ile Trp Ala Gly
            105                 110                 115

gca gag gat ggt agc tgt tct tca gag ccc ttg gat gag aag cct aca      500
Ala Glu Asp Gly Ser Cys Ser Ser Glu Pro Leu Asp Glu Lys Pro Thr
        120                 125                 130

gta gtg aag gtc ctt cgc aga tat gca agg agg agg cag ctg aag gtg      548
Val Val Lys Val Leu Arg Arg Tyr Ala Arg Arg Arg Gln Leu Lys Val
    135                 140                 145

gta gca gac ctg tgc ctc agg ccc cgc cat gat gaa aca caa gca tac      596
Val Ala Asp Leu Cys Leu Arg Pro Arg His Asp Glu Thr Gln Ala Tyr
150                 155                 160                 165

ttc ttg aag tgg gcc cag cag aga aag gac tcc cta cat ttg tgc tgt      644
Phe Leu Lys Trp Ala Gln Gln Arg Lys Asp Ser Leu His Leu Cys Cys
                170                 175                 180

ata aac atg aag atc tgg gct atg ccc gtg gac ttt gtc tta gag att      692
Ile Asn Met Lys Ile Trp Ala Met Pro Val Asp Phe Val Leu Glu Ile
            185                 190                 195

ttg aat gtc ttt cat cca gag cac atc gag gaa ttc gaa ctg aac act      740
Leu Asn Val Phe His Pro Glu His Ile Glu Glu Phe Glu Leu Asn Thr
        200                 205                 210

gag tgg aat gtg ttc aat ctg gcc cgt ttt gct ccc tgc tta tgg cag      788
Glu Trp Asn Val Phe Asn Leu Ala Arg Phe Ala Pro Cys Leu Trp Gln
    215                 220                 225
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atg aga aat ctt cgc aaa ctt ctc ctg gca ccc ctc tat aag aat gtc      836
Met Arg Asn Leu Arg Lys Leu Leu Leu Ala Pro Leu Tyr Lys Asn Val
230                 235                 240                 245

ttc aag att gcc aat agg aca gga gac aga gaa gat aag tgt gtc aag      884
Phe Lys Ile Ala Asn Arg Thr Gly Asp Arg Glu Asp Lys Cys Val Lys
                250                 255                 260

gag ttc gtt tct atc ttc tcc aaa ttc aat tgt ctc cag cat ctc tcc      932
Glu Phe Val Ser Ile Phe Ser Lys Phe Asn Cys Leu Gln His Leu Ser
            265                 270                 275

atg caa ggt gtc cac ttt ctc aca gac cac atg agt cag gtc ttc agg      980
Met Gln Gly Val His Phe Leu Thr Asp His Met Ser Gln Val Phe Arg
        280                 285                 290

tgc ttg atg aca ccc ttg ggg tcc ctc tcc atc act cac tac caa att     1028
Cys Leu Met Thr Pro Leu Gly Ser Leu Ser Ile Thr His Tyr Gln Ile
    295                 300                 305

tca cag tca gac ttg gat tcc ttc tct tgc tgt cag agt ctc ttt cag     1076
Ser Gln Ser Asp Leu Asp Ser Phe Ser Cys Cys Gln Ser Leu Phe Gln
310                 315                 320                 325

cta aat cat ctg gag atg aaa ggc gtg gtc tta cag gtt ttg gat gtg     1124
Leu Asn His Leu Glu Met Lys Gly Val Val Leu Gln Val Leu Asp Val
                330                 335                 340

atg cct ctg aga ggt ctc tta gag aaa gtg gta aaa act ctt gag act     1172
Met Pro Leu Arg Gly Leu Leu Glu Lys Val Val Lys Thr Leu Glu Thr
            345                 350                 355

ctg aat ttg cag gga tgt aag ctg aag gac tct cag ctc aat gca ctc     1220
Leu Asn Leu Gln Gly Cys Lys Leu Lys Asp Ser Gln Leu Asn Ala Leu
        360                 365                 370

cta cct tcc ttc ata caa tgc tct cag ctc acc aag gtc aac ttt tac     1268
Leu Pro Ser Phe Ile Gln Cys Ser Gln Leu Thr Lys Val Asn Phe Tyr
    375                 380                 385

aac aat gac ttc tcc atg ccc atc ctg aag gac ctt tta cag cac aca     1316
Asn Asn Asp Phe Ser Met Pro Ile Leu Lys Asp Leu Leu Gln His Thr
390                 395                 400                 405

gcc aac tgg aac aag atg aat gtg gaa cag tac cct gcc tct ctg gag     1364
Ala Asn Trp Asn Lys Met Asn Val Glu Gln Tyr Pro Ala Ser Leu Glu
                410                 415                 420

tgc tat aat gag ttg gga cat gtc tct gta gaa aga ttt gcc caa ctt     1412
Cys Tyr Asn Glu Leu Gly His Val Ser Val Glu Arg Phe Ala Gln Leu
            425                 430                 435

tgt cag gaa ctc atg gat aca cta agg gca ata agg cag ccc aag agc     1460
Cys Gln Glu Leu Met Asp Thr Leu Arg Ala Ile Arg Gln Pro Lys Ser
        440                 445                 450

ctc tct ttt gct aca cgt ata tgc cac aaa tgt ggt gag tgc tgt gtc     1508
Leu Ser Phe Ala Thr Arg Ile Cys His Lys Cys Gly Glu Cys Cys Val
    455                 460                 465

tat ggc aag aga gcc aga ctt tgt ttt tgc tgg cgg tga acatggattc      1557
Tyr Gly Lys Arg Ala Arg Leu Cys Phe Cys Trp Arg
470                 475                 480

agaacttctg catgtgaata aatgacagtc ttgagacgca aaaaaaaaaa aaaaaaaaaa   1617

aaaaaaaa                                                           1625


<210> SEQ ID NO 37
<211> LENGTH: 481
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 37

Met Ser Val Gln Thr Leu Ser Thr Leu Gln Asn Leu Thr Leu Lys Ala
1               5                   10                  15

Leu Leu Arg Asp Glu Ala Leu Ala Leu Ser Cys Leu Glu Glu Val Pro
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           20                  25                  30

Phe Leu Leu Phe Pro Ala Leu Phe Gln Arg Ala Phe Ala Gly Arg Leu
        35                  40                  45

Lys Lys Leu Met Lys Ala Ile Met Ala Ala Trp Thr Phe Pro Cys Leu
    50                  55                  60

Pro Val Gly Ala Leu Met Lys Ser Pro Asn Leu Glu Thr Leu Gln Ala
65                  70                  75                  80

Val Leu Asp Gly Ile Asp Met Gln Leu Thr Arg Glu Ser His Pro Arg
                85                  90                  95

Gly Lys Leu Gln Val Leu Asp Leu Arg Asn Val His His Ala Phe Trp
            100                 105                 110

Asp Ile Trp Ala Gly Ala Glu Asp Gly Ser Cys Ser Ser Glu Pro Leu
        115                 120                 125

Asp Glu Lys Pro Thr Val Val Lys Val Leu Arg Arg Tyr Ala Arg Arg
    130                 135                 140

Arg Gln Leu Lys Val Val Ala Asp Leu Cys Leu Arg Pro Arg His Asp
145                 150                 155                 160

Glu Thr Gln Ala Tyr Phe Leu Lys Trp Ala Gln Gln Arg Lys Asp Ser
                165                 170                 175

Leu His Leu Cys Cys Ile Asn Met Lys Ile Trp Ala Met Pro Val Asp
            180                 185                 190

Phe Val Leu Glu Ile Leu Asn Val Phe His Pro Glu His Ile Glu Glu
        195                 200                 205

Phe Glu Leu Asn Thr Glu Trp Asn Val Phe Asn Leu Ala Arg Phe Ala
    210                 215                 220

Pro Cys Leu Trp Gln Met Arg Asn Leu Arg Lys Leu Leu Leu Ala Pro
225                 230                 235                 240

Leu Tyr Lys Asn Val Phe Lys Ile Ala Asn Arg Thr Gly Asp Arg Glu
                245                 250                 255

Asp Lys Cys Val Lys Glu Phe Val Ser Ile Phe Ser Lys Phe Asn Cys
            260                 265                 270

Leu Gln His Leu Ser Met Gln Gly Val His Phe Leu Thr Asp His Met
        275                 280                 285

Ser Gln Val Phe Arg Cys Leu Met Thr Pro Leu Gly Ser Leu Ser Ile
    290                 295                 300

Thr His Tyr Gln Ile Ser Gln Ser Asp Leu Asp Ser Phe Ser Cys Cys
305                 310                 315                 320

Gln Ser Leu Phe Gln Leu Asn His Leu Glu Met Lys Gly Val Val Leu
                325                 330                 335

Gln Val Leu Asp Val Met Pro Leu Arg Gly Leu Leu Glu Lys Val Val
            340                 345                 350

Lys Thr Leu Glu Thr Leu Asn Leu Gln Gly Cys Lys Leu Lys Asp Ser
        355                 360                 365

Gln Leu Asn Ala Leu Leu Pro Ser Phe Ile Gln Cys Ser Gln Leu Thr
    370                 375                 380

Lys Val Asn Phe Tyr Asn Asn Asp Phe Ser Met Pro Ile Leu Lys Asp
385                 390                 395                 400

Leu Leu Gln His Thr Ala Asn Trp Asn Lys Met Asn Val Glu Gln Tyr
                405                 410                 415

Pro Ala Ser Leu Glu Cys Tyr Asn Glu Leu Gly His Val Ser Val Glu
            420                 425                 430

Arg Phe Ala Gln Leu Cys Gln Glu Leu Met Asp Thr Leu Arg Ala Ile
        435                 440                 445
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Arg Gln Pro Lys Ser Leu Ser Phe Ala Thr Arg Ile Cys His Lys Cys
    450                 455                 460

Gly Glu Cys Cys Val Tyr Gly Lys Arg Ala Arg Leu Cys Phe Cys Trp
465                 470                 475                 480

Arg
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<210> SEQ ID NO 38
<211> LENGTH: 1325
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (120)..(1202)

<400> SEQUENCE: 38

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acttgtagta gtccagggaa gtaagcagag ctccttgcac tgcagactct tgtgaacacc     60

gggacacatt agaccctagt ttcctcactg tgttcgggaa aggaagctca ggagacaaa     119

atg cag aga gaa gat aac cga gtc caa agt gtg aga aat gac aaa gaa      167
Met Gln Arg Glu Asp Asn Arg Val Gln Ser Val Arg Asn Asp Lys Glu
1               5                   10                  15

gcc aat agg agg agg agg ctg agg caa gaa ggc caa agt tcc tca ggt      215
Ala Asn Arg Arg Arg Arg Leu Arg Gln Glu Gly Gln Ser Ser Ser Gly
            20                  25                  30

ccg tgt gat agc ccg tgg act gag gat gaa atc tgg atc ttg ctg caa      263
Pro Cys Asp Ser Pro Trp Thr Glu Asp Glu Ile Trp Ile Leu Leu Gln
        35                  40                  45

gag tgg gca atg gtt gaa tat gaa ctc gga gac cca ggc aat aag atg      311
Glu Trp Ala Met Val Glu Tyr Glu Leu Gly Asp Pro Gly Asn Lys Met
    50                  55                  60

cat gcg aag gcc aag tcc ctt agc aga cgc ctc tct aat cgg ggt ctg      359
His Ala Lys Ala Lys Ser Leu Ser Arg Arg Leu Ser Asn Arg Gly Leu
65                  70                  75                  80

agg aag agc aag aat agc tgc ctt gat gtg atg gtg aag atg aag gac      407
Arg Lys Ser Lys Asn Ser Cys Leu Asp Val Met Val Lys Met Lys Asp
                85                  90                  95

ctg cac aca cgt ctt tgt aac gag agg ccc cgg gct tac cgc ttg tat      455
Leu His Thr Arg Leu Cys Asn Glu Arg Pro Arg Ala Tyr Arg Leu Tyr
            100                 105                 110

tcg act tat gaa tgg atc ctg tac gag atc ttg ggc cac ccc aga tcc      503
Ser Thr Tyr Glu Trp Ile Leu Tyr Glu Ile Leu Gly His Pro Arg Ser
        115                 120                 125

cag gga ggc tat gtg cca ggt cct tgg ttt gat ggg cac ggt aac cca      551
Gln Gly Gly Tyr Val Pro Gly Pro Trp Phe Asp Gly His Gly Asn Pro
    130                 135                 140

cca gct tcc tat gca act tcc ctc tgc att ggt ggt gcc atc tct cta      599
Pro Ala Ser Tyr Ala Thr Ser Leu Cys Ile Gly Gly Ala Ile Ser Leu
145                 150                 155                 160

ggc cct tcc ttt agc cca tgg acc gac cct gaa atc aag atc ttc ctg      647
Gly Pro Ser Phe Ser Pro Trp Thr Asp Pro Glu Ile Lys Ile Phe Leu
                165                 170                 175

cag gag tgg caa gtg gtt gaa cgg gaa ttt ggc cac cca ggc cag aag      695
Gln Glu Trp Gln Val Val Glu Arg Glu Phe Gly His Pro Gly Gln Lys
            180                 185                 190

atc aag cag aag agc agt ctt gtt tgc cag cgt ctc tat cat cga ggc      743
Ile Lys Gln Lys Ser Ser Leu Val Cys Gln Arg Leu Tyr His Arg Gly
        195                 200                 205

ctg ttc aag gac atc caa agc tgt ttg gac ctg atg tgg acc atg aag      791
Leu Phe Lys Asp Ile Gln Ser Cys Leu Asp Leu Met Trp Thr Met Lys
    210                 215                 220

gat ctg cac tcc act ctc agt aga gag aga tca agg act gta ccc ttg      839
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Asp Leu His Ser Thr Leu Ser Arg Glu Arg Ser Arg Thr Val Pro Leu
225                 230                 235                 240

ttt tct cct tat aga gat tat ctg gaa agg atc ttc gac ccc aaa tgt      887
Phe Ser Pro Tyr Arg Asp Tyr Leu Glu Arg Ile Phe Asp Pro Lys Cys
                245                 250                 255

cag aga ggc cat gtt cca ggt gtt cag tat aat tgg tct ggt tac cac      935
Gln Arg Gly His Val Pro Gly Val Gln Tyr Asn Trp Ser Gly Tyr His
            260                 265                 270

agg cct tcc tca aac cct caa act cca atg gtg atg cca tct cct gta      983
Arg Pro Ser Ser Asn Pro Gln Thr Pro Met Val Met Pro Ser Pro Val
        275                 280                 285

tac cag cct tgg gat tat ggc atg gct gca tct tct ggt cag ctt ccc     1031
Tyr Gln Pro Trp Asp Tyr Gly Met Ala Ala Ser Ser Gly Gln Leu Pro
    290                 295                 300

tgg atc cca tta cta atc atg tcc agt cag gac tta ctg gtt ccc aga     1079
Trp Ile Pro Leu Leu Ile Met Ser Ser Gln Asp Leu Leu Val Pro Arg
305                 310                 315                 320

tgg gat gcc tgg aat gcc acc tat cca ttg cca gtt caa cat gta ttt     1127
Trp Asp Ala Trp Asn Ala Thr Tyr Pro Leu Pro Val Gln His Val Phe
                325                 330                 335

cag gcc tct ctc cct gga gac aac aac ttt cag cag ctg tgg tca cct     1175
Gln Ala Ser Leu Pro Gly Asp Asn Asn Phe Gln Gln Leu Trp Ser Pro
            340                 345                 350

cgt gat gag agc tca agt cct cag tga agacatgtgg ggacttttct          1222
Arg Asp Glu Ser Ser Ser Pro Gln
        355                 360

ttttcctctg aaaaccacta agaatcttcc agcactgtat ggatcctcaa tgtctctatt   1282

ttattgtaaa ggaaatgtga aatcaaataa attattttga cac                     1325


<210> SEQ ID NO 39
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 39

Met Gln Arg Glu Asp Asn Arg Val Gln Ser Val Arg Asn Asp Lys Glu
1               5                   10                  15

Ala Asn Arg Arg Arg Arg Leu Arg Gln Glu Gly Gln Ser Ser Ser Gly
            20                  25                  30

Pro Cys Asp Ser Pro Trp Thr Glu Asp Glu Ile Trp Ile Leu Leu Gln
        35                  40                  45

Glu Trp Ala Met Val Glu Tyr Glu Leu Gly Asp Pro Gly Asn Lys Met
    50                  55                  60

His Ala Lys Ala Lys Ser Leu Ser Arg Arg Leu Ser Asn Arg Gly Leu
65                  70                  75                  80

Arg Lys Ser Lys Asn Ser Cys Leu Asp Val Met Val Lys Met Lys Asp
                85                  90                  95

Leu His Thr Arg Leu Cys Asn Glu Arg Pro Arg Ala Tyr Arg Leu Tyr
            100                 105                 110

Ser Thr Tyr Glu Trp Ile Leu Tyr Glu Ile Leu Gly His Pro Arg Ser
        115                 120                 125

Gln Gly Gly Tyr Val Pro Gly Pro Trp Phe Asp Gly His Gly Asn Pro
    130                 135                 140

Pro Ala Ser Tyr Ala Thr Ser Leu Cys Ile Gly Gly Ala Ile Ser Leu
145                 150                 155                 160

Gly Pro Ser Phe Ser Pro Trp Thr Asp Pro Glu Ile Lys Ile Phe Leu
                165                 170                 175
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Gln Glu Trp Gln Val Val Glu Arg Glu Phe Gly His Pro Gly Gln Lys
            180                 185                 190

Ile Lys Gln Lys Ser Ser Leu Val Cys Gln Arg Leu Tyr His Arg Gly
        195                 200                 205

Leu Phe Lys Asp Ile Gln Ser Cys Leu Asp Leu Met Trp Thr Met Lys
    210                 215                 220

Asp Leu His Ser Thr Leu Ser Arg Glu Arg Ser Arg Thr Val Pro Leu
225                 230                 235                 240

Phe Ser Pro Tyr Arg Asp Tyr Leu Glu Arg Ile Phe Asp Pro Lys Cys
                245                 250                 255

Gln Arg Gly His Val Pro Gly Val Gln Tyr Asn Trp Ser Gly Tyr His
            260                 265                 270

Arg Pro Ser Ser Asn Pro Gln Thr Pro Met Val Met Pro Ser Pro Val
        275                 280                 285

Tyr Gln Pro Trp Asp Tyr Gly Met Ala Ala Ser Ser Gly Gln Leu Pro
    290                 295                 300

Trp Ile Pro Leu Leu Ile Met Ser Ser Gln Asp Leu Leu Val Pro Arg
305                 310                 315                 320

Trp Asp Ala Trp Asn Ala Thr Tyr Pro Leu Pro Val Gln His Val Phe
                325                 330                 335

Gln Ala Ser Leu Pro Gly Asp Asn Asn Phe Gln Gln Leu Trp Ser Pro
            340                 345                 350

Arg Asp Glu Ser Ser Ser Pro Gln
        355                 360


<210> SEQ ID NO 40
<211> LENGTH: 1415
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (81)..(1145)

<400> SEQUENCE: 40

agctgtggga ggctgcactc actcgaggtc ctgagttgca ccgagccggt tctcctaggg     60

taatcccctc cctgccaatc atg ttc ctg agg agc agc gcc tcc cgt ctc ctc    113
                     Met Phe Leu Arg Ser Ser Ala Ser Arg Leu Leu
                     1               5                   10

cac ggg caa att cct tgc gtc ctg acg aga tcc gtc cac tct gta gct      161
His Gly Gln Ile Pro Cys Val Leu Thr Arg Ser Val His Ser Val Ala
            15                  20                  25

ata gtc gga gcc cct ttc tct cgg gga cag aag aag cta gga gtg gaa      209
Ile Val Gly Ala Pro Phe Ser Arg Gly Gln Lys Lys Leu Gly Val Glu
        30                  35                  40

tat ggt cca gct gcc att cga gaa gct ggc ttg ctg aag agg ctc tcc      257
Tyr Gly Pro Ala Ala Ile Arg Glu Ala Gly Leu Leu Lys Arg Leu Ser
    45                  50                  55

agg ttg gga tgc cac cta aaa gac ttt gga gac ttg agt ttt act aat      305
Arg Leu Gly Cys His Leu Lys Asp Phe Gly Asp Leu Ser Phe Thr Asn
60                  65                  70                  75

gtc cca caa gat gat ccc tac aat aat ctg gtt gtg tat cct cgt tca      353
Val Pro Gln Asp Asp Pro Tyr Asn Asn Leu Val Val Tyr Pro Arg Ser
                80                  85                  90

gtg ggc ctt gcc aac cag gaa ctg gct gaa gtg gtt agt aga gct gtg      401
Val Gly Leu Ala Asn Gln Glu Leu Ala Glu Val Val Ser Arg Ala Val
            95                  100                 105

tca ggt ggc tac agc tgt gtc acc atg gga gga gac cac agc ctg gca      449
Ser Gly Gly Tyr Ser Cys Val Thr Met Gly Gly Asp His Ser Leu Ala
        110                 115                 120
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ata ggt acc att atc ggt cac gcc cgg cac cgc cca gat ctc tgt gtc      497
Ile Gly Thr Ile Ile Gly His Ala Arg His Arg Pro Asp Leu Cys Val
    125                 130                 135

atc tgg gtt gat gct cat gcg gac att aat aca cct ctc acc act gta      545
Ile Trp Val Asp Ala His Ala Asp Ile Asn Thr Pro Leu Thr Thr Val
140                 145                 150                 155

tct gga aat ata cat gga cag cca ctt tcc ttt ctc atc aaa gaa cta      593
Ser Gly Asn Ile His Gly Gln Pro Leu Ser Phe Leu Ile Lys Glu Leu
                160                 165                 170

caa gac aag gta cca caa ctg cca gga ttt tcc tgg atc aaa cct tgc      641
Gln Asp Lys Val Pro Gln Leu Pro Gly Phe Ser Trp Ile Lys Pro Cys
            175                 180                 185

ctc tct ccc cca aat att gtg tac att ggc ctg aga gat gtg gag cct      689
Leu Ser Pro Pro Asn Ile Val Tyr Ile Gly Leu Arg Asp Val Glu Pro
        190                 195                 200

cct gaa cat ttt att tta aag aat tat gac atc cag tat ttt tcc atg      737
Pro Glu His Phe Ile Leu Lys Asn Tyr Asp Ile Gln Tyr Phe Ser Met
    205                 210                 215

aga gag att gat cga ctt ggg atc cag aag gtg atg gaa cag aca ttt      785
Arg Glu Ile Asp Arg Leu Gly Ile Gln Lys Val Met Glu Gln Thr Phe
220                 225                 230                 235

gat cgg ctg att ggc aaa agg cag agg cca atc cac ctg agt ttt gac      833
Asp Arg Leu Ile Gly Lys Arg Gln Arg Pro Ile His Leu Ser Phe Asp
                240                 245                 250

att gat gca ttt gac cct aaa ctg gct cca gcc aca gga acc cct gtt      881
Ile Asp Ala Phe Asp Pro Lys Leu Ala Pro Ala Thr Gly Thr Pro Val
            255                 260                 265

gta ggg gga tta acc tac aga gaa gga gtg tat att act gaa gaa ata      929
Val Gly Gly Leu Thr Tyr Arg Glu Gly Val Tyr Ile Thr Glu Glu Ile
        270                 275                 280

cat aat aca ggg ttg ctg tca gct ctg gat ctt gtt gaa gtc aat cct      977
His Asn Thr Gly Leu Leu Ser Ala Leu Asp Leu Val Glu Val Asn Pro
    285                 290                 295

cat ttg gcc act tct gag gaa gag gcc aag gca aca gcc aga cta gca     1025
His Leu Ala Thr Ser Glu Glu Glu Ala Lys Ala Thr Ala Arg Leu Ala
300                 305                 310                 315

gtg gat gtg att gct tca agt ttt ggt cag aca aga gaa gga gga cac     1073
Val Asp Val Ile Ala Ser Ser Phe Gly Gln Thr Arg Glu Gly Gly His
                320                 325                 330

att gtc tat gac cac ctt cct act cct agt tca cca cac gaa tca gaa     1121
Ile Val Tyr Asp His Leu Pro Thr Pro Ser Ser Pro His Glu Ser Glu
            335                 340                 345

aat gaa gaa tgt gtg aga att tag gaaatactgt actctggcac ctttcacaac    1175
Asn Glu Glu Cys Val Arg Ile
        350

agcattacag agttgcaagg cattcgaagg gacagatatg aaatggctgt ctggatcaat   1235

attgccttaa tgagaacatc tgtgcactct cacaactgta aaactccctt ctctattttg   1295

gtcaccaaca ctattactgt aaatgtattt tttgttgttt ttgaagttta caagctatta   1355

atgttataca tgtaagtttg aaggagtcat aaacaacatt tattacctta gtatatcata   1415


<210> SEQ ID NO 41
<211> LENGTH: 354
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 41

Met Phe Leu Arg Ser Ser Ala Ser Arg Leu Leu His Gly Gln Ile Pro
1               5                   10                  15
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Cys Val Leu Thr Arg Ser Val His Ser Val Ala Ile Val Gly Ala Pro
            20                  25                  30

Phe Ser Arg Gly Gln Lys Lys Leu Gly Val Glu Tyr Gly Pro Ala Ala
        35                  40                  45

Ile Arg Glu Ala Gly Leu Leu Lys Arg Leu Ser Arg Leu Gly Cys His
    50                  55                  60

Leu Lys Asp Phe Gly Asp Leu Ser Phe Thr Asn Val Pro Gln Asp Asp
65                  70                  75                  80

Pro Tyr Asn Asn Leu Val Val Tyr Pro Arg Ser Val Gly Leu Ala Asn
                85                  90                  95

Gln Glu Leu Ala Glu Val Val Ser Arg Ala Val Ser Gly Gly Tyr Ser
            100                 105                 110

Cys Val Thr Met Gly Gly Asp His Ser Leu Ala Ile Gly Thr Ile Ile
        115                 120                 125

Gly His Ala Arg His Arg Pro Asp Leu Cys Val Ile Trp Val Asp Ala
    130                 135                 140

His Ala Asp Ile Asn Thr Pro Leu Thr Thr Val Ser Gly Asn Ile His
145                 150                 155                 160

Gly Gln Pro Leu Ser Phe Leu Ile Lys Glu Leu Gln Asp Lys Val Pro
                165                 170                 175

Gln Leu Pro Gly Phe Ser Trp Ile Lys Pro Cys Leu Ser Pro Pro Asn
            180                 185                 190

Ile Val Tyr Ile Gly Leu Arg Asp Val Glu Pro Pro Glu His Phe Ile
        195                 200                 205

Leu Lys Asn Tyr Asp Ile Gln Tyr Phe Ser Met Arg Glu Ile Asp Arg
    210                 215                 220

Leu Gly Ile Gln Lys Val Met Glu Gln Thr Phe Asp Arg Leu Ile Gly
225                 230                 235                 240

Lys Arg Gln Arg Pro Ile His Leu Ser Phe Asp Ile Asp Ala Phe Asp
                245                 250                 255

Pro Lys Leu Ala Pro Ala Thr Gly Thr Pro Val Val Gly Gly Leu Thr
            260                 265                 270

Tyr Arg Glu Gly Val Tyr Ile Thr Glu Glu Ile His Asn Thr Gly Leu
        275                 280                 285

Leu Ser Ala Leu Asp Leu Val Glu Val Asn Pro His Leu Ala Thr Ser
    290                 295                 300

Glu Glu Glu Ala Lys Ala Thr Ala Arg Leu Ala Val Asp Val Ile Ala
305                 310                 315                 320

Ser Ser Phe Gly Gln Thr Arg Glu Gly Gly His Ile Val Tyr Asp His
                325                 330                 335

Leu Pro Thr Pro Ser Ser Pro His Glu Ser Glu Asn Glu Glu Cys Val
            340                 345                 350

Arg Ile


<210> SEQ ID NO 42
<211> LENGTH: 858
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (151)..(666)

<400> SEQUENCE: 42

gcctgtgatt ccgtcttcta ctgaagacca cctgaaccat ccatcctcag gaactgagaa     60

cttctggaat cttggacttt acttcctctc cagctgttgt ggaataagta caactgcagc    120
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ctgaggtgga ggatttacct tcagggatcc atg gat aaa gcc aag aag atg atg     174
                               Met Asp Lys Ala Lys Lys Met Met
                               1               5

cag tcc att ccc agt ttt gtc aag gat aca tca gat att gaa gaa cat      222
Gln Ser Ile Pro Ser Phe Val Lys Asp Thr Ser Asp Ile Glu Glu His
    10                  15                  20

gca ctg ccc agt gca cag gtc ttg cca gcc cag agt aca agg tgt tct      270
Ala Leu Pro Ser Ala Gln Val Leu Pro Ala Gln Ser Thr Arg Cys Ser
25                  30                  35                  40

aat tct gag gca ctt tgt tta ggc aaa gat caa agc cac tgc tct gag      318
Asn Ser Glu Ala Leu Cys Leu Gly Lys Asp Gln Ser His Cys Ser Glu
                45                  50                  55

gat ggc tgg att gcc gaa tgg gat cta tac tcc ttt tgt gta ttt gag      366
Asp Gly Trp Ile Ala Glu Trp Asp Leu Tyr Ser Phe Cys Val Phe Glu
            60                  65                  70

agt gtg gac tac ctg aga tcc tac cga aga ttg aat tct gcc atg aag      414
Ser Val Asp Tyr Leu Arg Ser Tyr Arg Arg Leu Asn Ser Ala Met Lys
        75                  80                  85

aag ggc aca gag gtc ttc cag agt gag agt cag agg aag cca aaa gtg      462
Lys Gly Thr Glu Val Phe Gln Ser Glu Ser Gln Arg Lys Pro Lys Val
    90                  95                  100

tcc cca gga gat gtg gaa aac tac aaa gac aaa gat aca gag aag cca      510
Ser Pro Gly Asp Val Glu Asn Tyr Lys Asp Lys Asp Thr Glu Lys Pro
105                 110                 115                 120

gac caa ccc tcc cca agc ttg ctc agg gag aaa ggt ctg gat ctt gtg      558
Asp Gln Pro Ser Pro Ser Leu Leu Arg Glu Lys Gly Leu Asp Leu Val
                125                 130                 135

acc tgt gac ggt gga gac tgc cct gtc cgg gat cct gtt tct gac agt      606
Thr Cys Asp Gly Gly Asp Cys Pro Val Arg Asp Pro Val Ser Asp Ser
            140                 145                 150

tcc agg cac cta ggc tgc tgg gca tgg ttt caa agg gct ttt ggc cat      654
Ser Arg His Leu Gly Cys Trp Ala Trp Phe Gln Arg Ala Phe Gly His
        155                 160                 165

aag aag aag tga gaaaggcact aagaactgtg tttggagccc atgaaccctg          706
Lys Lys Lys
    170

atgcctgcta agacttgcaa ttaggggacc ttctgtcagc ttctgctgtt agagcaaagg    766

cacacaaagg cagttgtgtc tttgcagcca tctggtttgt gtttgtttgt ttatttgttt    826

acagcatttc ttaataaaat tgttaaaaag ct                                  858


<210> SEQ ID NO 43
<211> LENGTH: 171
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 43

Met Asp Lys Ala Lys Lys Met Met Gln Ser Ile Pro Ser Phe Val Lys
1               5                   10                  15

Asp Thr Ser Asp Ile Glu Glu His Ala Leu Pro Ser Ala Gln Val Leu
            20                  25                  30

Pro Ala Gln Ser Thr Arg Cys Ser Asn Ser Glu Ala Leu Cys Leu Gly
        35                  40                  45

Lys Asp Gln Ser His Cys Ser Glu Asp Gly Trp Ile Ala Glu Trp Asp
    50                  55                  60

Leu Tyr Ser Phe Cys Val Phe Glu Ser Val Asp Tyr Leu Arg Ser Tyr
65                  70                  75                  80

Arg Arg Leu Asn Ser Ala Met Lys Lys Gly Thr Glu Val Phe Gln Ser
                85                  90                  95
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Glu Ser Gln Arg Lys Pro Lys Val Ser Pro Gly Asp Val Glu Asn Tyr
            100                 105                 110

Lys Asp Lys Asp Thr Glu Lys Pro Asp Gln Pro Ser Pro Ser Leu Leu
        115                 120                 125

Arg Glu Lys Gly Leu Asp Leu Val Thr Cys Asp Gly Gly Asp Cys Pro
    130                 135                 140

Val Arg Asp Pro Val Ser Asp Ser Ser Arg His Leu Gly Cys Trp Ala
145                 150                 155                 160

Trp Phe Gln Arg Ala Phe Gly His Lys Lys Lys
                165                 170


<210> SEQ ID NO 44
<211> LENGTH: 876
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (182)..(691)

<400> SEQUENCE: 44

agtctatact tcgctggcac tagagcccct tgcatgtgat tccatcttct attgaagacc     60

agctgaaaca tccatcctca ggaactgaga acttctggaa tcttggactt tacttcctct    120

ccagctgttg tggaataagt tcaactccag actgaggtgg aggatttacc ttcagggatc    180

c atg gat aaa gcc aag aag atg atg cag tcc att ccc agt ttt gtc aag    229
  Met Asp Lys Ala Lys Lys Met Met Gln Ser Ile Pro Ser Phe Val Lys
  1               5                  10                  15

gat aca tca gat att gaa gaa cat gca ctg ccc agt gca cag gtc ttg      277
Asp Thr Ser Asp Ile Glu Glu His Ala Leu Pro Ser Ala Gln Val Leu
            20                  25                  30

cca gcc cag agt aca agg tgt tcc aat tct gag aca ctt tgt ttc agc      325
Pro Ala Gln Ser Thr Arg Cys Ser Asn Ser Glu Thr Leu Cys Phe Ser
        35                  40                  45

aaa gag caa agc cac tgc tct gag gat ggc tgg att gcc aat tgg gat      373
Lys Glu Gln Ser His Cys Ser Glu Asp Gly Trp Ile Ala Asn Trp Asp
    50                  55                  60

cta tac tcc ttt tgt gta ttt gag agt gtg gac tac ctg aaa tcc tac      421
Leu Tyr Ser Phe Cys Val Phe Glu Ser Val Asp Tyr Leu Lys Ser Tyr
65                  70                  75                  80

cgc aga ttg aat tct gcc atg aag aag ggc aca gag gtc ttc cag agt      469
Arg Arg Leu Asn Ser Ala Met Lys Lys Gly Thr Glu Val Phe Gln Ser
                85                  90                  95

gag agt cag agg gag cca caa gtg tcc cca gga gat gtg gaa aac tac      517
Glu Ser Gln Arg Glu Pro Gln Val Ser Pro Gly Asp Val Glu Asn Tyr
            100                 105                 110

aaa gac aaa gat aca gag gag cca gac caa ccc tca cta agc ttg ctc      565
Lys Asp Lys Asp Thr Glu Glu Pro Asp Gln Pro Ser Leu Ser Leu Leu
        115                 120                 125

agg gag aaa ggg ctg gaa ctt gtg acc tgt gat ggt gga gac tgc cct      613
Arg Glu Lys Gly Leu Glu Leu Val Thr Cys Asp Gly Gly Asp Cys Pro
    130                 135                 140

gac cag gat cct gca tct tat agt gcc agg cac cta ggc tgc tgg gca      661
Asp Gln Asp Pro Ala Ser Tyr Ser Ala Arg His Leu Gly Cys Trp Ala
145                 150                 155                 160

tgg ctt caa aga gct ttt cgc cag aag tga gaaagtcacc cagaactgtt        711
Trp Leu Gln Arg Ala Phe Arg Gln Lys
                165

tggatcccag attcctgcta agacttgcaa ttaggggatc ttctgtcagc tcctgctggt    771

acagcaaagg cacacaaagg cagttgtgtc ttttcagcca tctggtttgt gtttgtttgt    831
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ttgtttattt gtttgcagct ttcttaataa aattgttaaa aagct                    876


<210> SEQ ID NO 45
<211> LENGTH: 169
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 45

Met Asp Lys Ala Lys Lys Met Met Gln Ser Ile Pro Ser Phe Val Lys
1               5                   10                  15

Asp Thr Ser Asp Ile Glu Glu His Ala Leu Pro Ser Ala Gln Val Leu
            20                  25                  30

Pro Ala Gln Ser Thr Arg Cys Ser Asn Ser Glu Thr Leu Cys Phe Ser
        35                  40                  45

Lys Glu Gln Ser His Cys Ser Glu Asp Gly Trp Ile Ala Asn Trp Asp
    50                  55                  60

Leu Tyr Ser Phe Cys Val Phe Glu Ser Val Asp Tyr Leu Lys Ser Tyr
65                  70                  75                  80

Arg Arg Leu Asn Ser Ala Met Lys Lys Gly Thr Glu Val Phe Gln Ser
                85                  90                  95

Glu Ser Gln Arg Glu Pro Gln Val Ser Pro Gly Asp Val Glu Asn Tyr
            100                 105                 110

Lys Asp Lys Asp Thr Glu Glu Pro Asp Gln Pro Ser Leu Ser Leu Leu
        115                 120                 125

Arg Glu Lys Gly Leu Glu Leu Val Thr Cys Asp Gly Gly Asp Cys Pro
    130                 135                 140

Asp Gln Asp Pro Ala Ser Tyr Ser Ala Arg His Leu Gly Cys Trp Ala
145                 150                 155                 160

Trp Leu Gln Arg Ala Phe Arg Gln Lys
                165


<210> SEQ ID NO 46
<211> LENGTH: 811
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(492)

<400> SEQUENCE: 46

atg gct gac aaa atg gac atg tca ttg gaa gac atc att aag ctg atc       48
Met Ala Asp Lys Met Asp Met Ser Leu Glu Asp Ile Ile Lys Leu Ile
1               5                   10                  15

ttg tca aat ctg cac ttc gga gtg tca gat gct gat att cag cta ctc       96
Leu Ser Asn Leu His Phe Gly Val Ser Asp Ala Asp Ile Gln Leu Leu
            20                  25                  30

ttt gct gaa ttt gga acg ttg aag aaa tct gct gtg cac tat gat cgc      144
Phe Ala Glu Phe Gly Thr Leu Lys Lys Ser Ala Val His Tyr Asp Arg
        35                  40                  45

tgt gga cga agt tta ggg aca gca cag gtg cac ttt gaa agg aaa gca      192
Cys Gly Arg Ser Leu Gly Thr Ala Gln Val His Phe Glu Arg Lys Ala
    50                  55                  60

gat gcc ctg aag gct atg aga gag tac aat ggc gcc cct ttg gat ggc      240
Asp Ala Leu Lys Ala Met Arg Glu Tyr Asn Gly Ala Pro Leu Asp Gly
65                  70                  75                  80

cgc cct atg aac atc cag ctt gcc acc tca cag att gat aga caa gga      288
Arg Pro Met Asn Ile Gln Leu Ala Thr Ser Gln Ile Asp Arg Gln Gly
                85                  90                  95

aga cct gca caa agc aaa aat agg ggc ggc atg aca aga aac cct ggc      336
Arg Pro Ala Gln Ser Lys Asn Arg Gly Gly Met Thr Arg Asn Pro Gly
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            100                 105                 110

tct gga gta tta agt ggt gga ggc acc aag aaa tgg aca ctt gga ggc      384
Ser Gly Val Leu Ser Gly Gly Gly Thr Lys Lys Trp Thr Leu Gly Gly
        115                 120                 125

agc cag gga aga ggg aga ggc acc atc agg aac tca aag cag cag cta      432
Ser Gln Gly Arg Gly Arg Gly Thr Ile Arg Asn Ser Lys Gln Gln Leu
    130                 135                 140

tct gca gag gag ctg gat gcc cag ctg gat gct tat cag gaa atg atg      480
Ser Ala Glu Glu Leu Asp Ala Gln Leu Asp Ala Tyr Gln Glu Met Met
145                 150                 155                 160

gac acc agc tga acaattgagc aaagctgcac aagaacggaa cccatggcct          532
Asp Thr Ser

ggtctgtgat gcctagactg agggttggct actggaccat gaacacaatg gtggattcct    592

cctttgcttc ttttgctttt ctcctgtttt aaaaccccat gtaaagttct ttctttctct    652

ccttctttct tttatttaca ttcagaaata cacctgtttt gtgctgagtt attttgtgga    712

taaattatag tttttgcttt tgtgaagttg gcattttcac ctttgcccta ataaaattgt    772

gtgtagaaat aaacaagtat tctggagtca taaagtaat                          811


<210> SEQ ID NO 47
<211> LENGTH: 163
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 47

Met Ala Asp Lys Met Asp Met Ser Leu Glu Asp Ile Ile Lys Leu Ile
1               5                   10                  15

Leu Ser Asn Leu His Phe Gly Val Ser Asp Ala Asp Ile Gln Leu Leu
            20                  25                  30

Phe Ala Glu Phe Gly Thr Leu Lys Lys Ser Ala Val His Tyr Asp Arg
        35                  40                  45

Cys Gly Arg Ser Leu Gly Thr Ala Gln Val His Phe Glu Arg Lys Ala
    50                  55                  60

Asp Ala Leu Lys Ala Met Arg Glu Tyr Asn Gly Ala Pro Leu Asp Gly
65                  70                  75                  80

Arg Pro Met Asn Ile Gln Leu Ala Thr Ser Gln Ile Asp Arg Gln Gly
                85                  90                  95

Arg Pro Ala Gln Ser Lys Asn Arg Gly Gly Met Thr Arg Asn Pro Gly
            100                 105                 110

Ser Gly Val Leu Ser Gly Gly Gly Thr Lys Lys Trp Thr Leu Gly Gly
        115                 120                 125

Ser Gln Gly Arg Gly Arg Gly Thr Ile Arg Asn Ser Lys Gln Gln Leu
    130                 135                 140

Ser Ala Glu Glu Leu Asp Ala Gln Leu Asp Ala Tyr Gln Glu Met Met
145                 150                 155                 160

Asp Thr Ser


<210> SEQ ID NO 48
<211> LENGTH: 2881
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (354)..(788)

<400> SEQUENCE: 48

ggaaaggggc gtggccggcc gttgcctagg aagggcgcgt cgtctctctg ctcgtccggc     60
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tgtgacgggg aaggggtccc gctgcgtttt ggtcactact caggaggaga ccacaccttc    120

cggagaacca ggccagaacc gaagtactat tttgtagctc tcagaagcca ggactctgca    180

acactgtttg ctgcctgtgg atcttctata ttcacagtgt cccagttgct tctgatctac    240

cactgttaga tacttctgcc acccatccta agagtatagt tgttcttgga aaggagtctc    300

agctgctgtc agcaggagtc cctcattcga ctcctgtggt tgccctttcc atc atg       356
                                                          Met
                                                          1

cca aag aat aaa ggc aaa gga ggc aaa aac agg cgc aga ggt aaa aat      404
Pro Lys Asn Lys Gly Lys Gly Gly Lys Asn Arg Arg Arg Gly Lys Asn
            5                  10                  15

gaa aat gaa tct gag aaa aga gag ttg gtg ttt aaa gag gat ggg cag      452
Glu Asn Glu Ser Glu Lys Arg Glu Leu Val Phe Lys Glu Asp Gly Gln
        20                  25                  30

gag tat gct cag gtg atc aaa atg ctg gga aat gga cgg ttg gaa gca      500
Glu Tyr Ala Gln Val Ile Lys Met Leu Gly Asn Gly Arg Leu Glu Ala
    35                  40                  45

atg tgc ttt gac ggt gtg agg agg ctg tgc cat ata aga ggg aag ctg      548
Met Cys Phe Asp Gly Val Arg Arg Leu Cys His Ile Arg Gly Lys Leu
50                  55                  60                  65

aga aaa aag gtt tgg ata aat acc tcg gac att ata ttg att ggt cta      596
Arg Lys Lys Val Trp Ile Asn Thr Ser Asp Ile Ile Leu Ile Gly Leu
                70                  75                  80

cga gac tat caa gat aac aaa gct gat gta atc tta aag tat aat gca      644
Arg Asp Tyr Gln Asp Asn Lys Ala Asp Val Ile Leu Lys Tyr Asn Ala
            85                  90                  95

gat gaa gca aga agt ctg aag gcc tgt gga gaa ctt cca gaa cat gcc      692
Asp Glu Ala Arg Ser Leu Lys Ala Cys Gly Glu Leu Pro Glu His Ala
        100                 105                 110

aaa atc aat gaa acg gac aca ttt ggt cct ggg gat gat gat gaa atc      740
Lys Ile Asn Glu Thr Asp Thr Phe Gly Pro Gly Asp Asp Asp Glu Ile
    115                 120                 125

caa ttt gat gat att gga gat gat gat gaa gac att gat gac atc tag      788
Gln Phe Asp Asp Ile Gly Asp Asp Asp Glu Asp Ile Asp Asp Ile
130                 135                 140

cctgacctaa gccatgctac cttccaagtt gtctgaagat agctccacac agtggcatct    848

tgaccttcat ctgttaagta aaacttcatg gcatgtgtat gacttgttaa tgcaaggtaa    908

tgaatttttat tttttgaagt actatatttc tttgaaaacc aaagatgttg agttatcatc    968

ttaagtgaca tgttaacact ttgtgctttt gaatataatt gaacctagcg cacagcagtg   1028

agcactgtta agagactgcc tttccatttg tagcttcatt tctggcacgg gagtgttttg   1088

tgtcagcagt tctgccaggt ggccatcgtg aggctgaagt aagtcctagt ccagcacatc   1148

tgcttcaggc ctttgtactc tagtcatctg gctgcgttcg agacttctca gcagacttat   1208

agatgtgtac ggctgcactt ggagtcagac aagatatggc tacttttgta cttatggagc   1268

catgccattt tatactttca cgttgtatac attcgtttga tcctttaagt tgttgccacc   1328

cataaaaagg catcttacag tgcagttttt aaattacatg ggtagcaatt ttgagtttta   1388

aaaattagtc attgcagaaa ttaaatactt agaggagata atccattatc ttgactttag   1448

gaatataata gttgacaatg tttatatata attttacttc tctaaggcat acccaaaaat   1508

agaaaatgaa aaagagcagt gagtctgttc tgatgcttgc attgcataga gaagttttcc   1568

aacaaagcag ctgttaataa cacataaaat atgttttact ttgcaaagta ggttgtgtta   1628

agtcattttc aaaaagttac ctactatatc gaggctctgg ataattacta tgtgttgatt   1688

aaagttagtt acagaattgt acaagctaag ttttccttaa actaagctta ggttaaaggg   1748
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agaggagcca cagctcaatg aaaacacggt tcctgttttc taaatggagg cgcccagaaa   1808

cacaataaaa catgttggta caaaaacttt ttctttttaa tatgttcatt gtatctctgg   1868

tatataacaa aaataaatga ctgggtgatt tctggtatat catgagaggc ttttttttt   1928

ttttttaaa ttagactctg ggatttaaat gggacttaac tattttccca tttaaatgac   1988

gccagtattg gggtcctgca gcctaaccct gctgcttagg gagtgagtat aaaccgcgac   2048

tgtcagtcct cagatgcctt ccttttaaa gactagttct ttctcaggtc ttcttcttga   2108

cacctacaaa tggtgcctga ccacaagacg acagtattca tcttcacttt tatttttga   2168

ttgcttgttt tctagttaac ccagaataat atagcttatg aaaatctccc agtcaggaag   2228

aaagaaagaa agagaaagaa aagcaaatat gattttcctg atcattgatt ggtggatctc   2288

ttctagatgg agatatgtag atctttgtaa aggttaattt tataaagtga gagtagacat   2348

ggtacccaca cttagaagca gatcccacat ccccagaagg acagtgtgtg tttagaaaga   2408

acacatcact ggagcttttt attgctctac acagtgtatc taaataagct gtcaactaca   2468

atttatccta ttgctgctgt aaatttttat gacagaaaga aaacctgacc atggaccagc   2528

tagcttgatg gccttcagca gcaaacaaga aactgtccaa gttaggaggt gaggactagt   2588

gcctgaagat gtcctctcag tccacaacat gtacaggtgc ccatacacac atcagcactc   2648

gcacaaagat gctctggagg ctatagtagt gtgtcttggt cattgcaaac catcagaggc   2708

aaaccctgag gtattcccat ttcctgtttc ctgcttgcag tgtctacatt tctctcccat   2768

tctaatgaag gaatgatcct tttataacat gagtgatttt atgctgttta tagaagtaaa   2828

tgttgacatg tgttagaatt aaaatgactt agagaacctg aaaaaaaaaa acc          2881
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<210> SEQ ID NO 49
<211> LENGTH: 144
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 49

```
Met Pro Lys Asn Lys Gly Lys Gly Gly Lys Asn Arg Arg Arg Gly Lys
1               5                   10                  15

Asn Glu Asn Glu Ser Glu Lys Arg Glu Leu Val Phe Lys Glu Asp Gly
            20                  25                  30

Gln Glu Tyr Ala Gln Val Ile Lys Met Leu Gly Asn Gly Arg Leu Glu
        35                  40                  45

Ala Met Cys Phe Asp Gly Val Arg Arg Leu Cys His Ile Arg Gly Lys
    50                  55                  60

Leu Arg Lys Lys Val Trp Ile Asn Thr Ser Asp Ile Ile Leu Ile Gly
65                  70                  75                  80

Leu Arg Asp Tyr Gln Asp Asn Lys Ala Asp Val Ile Leu Lys Tyr Asn
                85                  90                  95

Ala Asp Glu Ala Arg Ser Leu Lys Ala Cys Gly Glu Leu Pro Glu His
            100                 105                 110

Ala Lys Ile Asn Glu Thr Asp Thr Phe Gly Pro Gly Asp Asp Asp Glu
        115                 120                 125

Ile Gln Phe Asp Asp Ile Gly Asp Asp Asp Glu Asp Ile Asp Asp Ile
    130                 135                 140
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<210> SEQ ID NO 50
<211> LENGTH: 1918
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS

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<222> LOCATION: (275)..(1918)

<400> SEQUENCE: 50

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attttgctct cggcttgcta gctagtgtac tccttctctg gcatcagagc ctactctttt     60

gggattccag ctcttactga agaccagctg agacattgac tgagcaactt tggattcttg    120

gactttccat tcatagacag acgtcactgg attagcaaga gcccatccta atctttggga    180

gacctgaggt acttccaacc caaaggactg ggcttcagga tttgcaaaca tcagctgtca    240

gctccttgcc tagcccaagg aatcctttgc caca atg tcc tgt gtg cac tac aaa    295
                                      Met Ser Cys Val His Tyr Lys
                                      1               5

ttt tcc tct aaa ctc agc tac aac acc atc acc ttt gat ggg ctc cat      343
Phe Ser Ser Lys Leu Ser Tyr Asn Thr Ile Thr Phe Asp Gly Leu His
        10                  15                  20

atc tcc ctc ttc tac tta aag aag cag att atg ggg aga gaa aag ctg      391
Ile Ser Leu Phe Tyr Leu Lys Lys Gln Ile Met Gly Arg Glu Lys Leu
    25                  30                  35

aaa act ggc aat agt gat ctg cag atc atc aat gca gag acg gaa gaa      439
Lys Thr Gly Asn Ser Asp Leu Gln Ile Ile Asn Ala Glu Thr Glu Glu
40                  45                  50                  55

gaa tat act gac gat aat gcg ctc atc cct aag aat tca tct gtg att      487
Glu Tyr Thr Asp Asp Asn Ala Leu Ile Pro Lys Asn Ser Ser Val Ile
                60                  65                  70

gtc aga aga att cct gtt gta ggt gtg aag tct aaa agc aag aca tat      535
Val Arg Arg Ile Pro Val Val Gly Val Lys Ser Lys Ser Lys Thr Tyr
            75                  80                  85

caa ata agt cac act aaa tca gtg atg gga act aca aga gca gtt aat      583
Gln Ile Ser His Thr Lys Ser Val Met Gly Thr Thr Arg Ala Val Asn
        90                  95                  100

gac tct tct gca ccg atg tct ctg gcc cag ctt ata gag act gcc aat      631
Asp Ser Ser Ala Pro Met Ser Leu Ala Gln Leu Ile Glu Thr Ala Asn
    105                 110                 115

ctg gct gag gcc aat gct tca gag gaa gac aaa att aaa gca atg atg      679
Leu Ala Glu Ala Asn Ala Ser Glu Glu Asp Lys Ile Lys Ala Met Met
120                 125                 130                 135

ata caa tct ggc cat gaa tat gac cca atc aat tac atg aag aaa act      727
Ile Gln Ser Gly His Glu Tyr Asp Pro Ile Asn Tyr Met Lys Lys Thr
                140                 145                 150

cca gta ggc ttg cca cct cca tct tac acc tgc ttt cgt tgt ggt aaa      775
Pro Val Gly Leu Pro Pro Pro Ser Tyr Thr Cys Phe Arg Cys Gly Lys
            155                 160                 165

cct ggt cat tat act aag aat tgc cca aca agt gtg aat aag gac ttt      823
Pro Gly His Tyr Thr Lys Asn Cys Pro Thr Ser Val Asn Lys Asp Phe
        170                 175                 180

gaa tct tgt cct agg atc aga aag agc act gga att cct aga aat ttt      871
Glu Ser Cys Pro Arg Ile Arg Lys Ser Thr Gly Ile Pro Arg Asn Phe
    185                 190                 195

atg atg gaa gtg aaa gat cct aac atg aaa ggt gca atg ctt aca aaa      919
Met Met Glu Val Lys Asp Pro Asn Met Lys Gly Ala Met Leu Thr Lys
200                 205                 210                 215

act ggg caa tat gca ata ccg act ata aat gca gag gcc tat gca att      967
Thr Gly Gln Tyr Ala Ile Pro Thr Ile Asn Ala Glu Ala Tyr Ala Ile
                220                 225                 230

ggg aag aaa agg aaa cca ccc ttc tta cca ggg gaa cct tca tca tca     1015
Gly Lys Lys Arg Lys Pro Pro Phe Leu Pro Gly Glu Pro Ser Ser Ser
            235                 240                 245

tct tca gaa gaa gtt ggt cct gtc cca gaa gag ctc ttg tgc ctc atc     1063
Ser Ser Glu Glu Val Gly Pro Val Pro Glu Glu Leu Leu Cys Leu Ile
        250                 255                 260
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tgc aag gac acc atg act gat gct gct atc atc ccc tgc tgt gga aac     1111
Cys Lys Asp Thr Met Thr Asp Ala Ala Ile Ile Pro Cys Cys Gly Asn
    265                 270                 275

agt tac tgt gat gaa tgt ata aga aca gca ctt ctg gag tca gat gaa     1159
Ser Tyr Cys Asp Glu Cys Ile Arg Thr Ala Leu Leu Glu Ser Asp Glu
280                 285                 290                 295

cat aca tgt cca aca tgt cat caa aat gat gtt tct cct gat gct tta     1207
His Thr Cys Pro Thr Cys His Gln Asn Asp Val Ser Pro Asp Ala Leu
                300                 305                 310

gtt gcc aac aag gtt tta cga cag gct gtt aat aac ttt aaa aat caa     1255
Val Ala Asn Lys Val Leu Arg Gln Ala Val Asn Asn Phe Lys Asn Gln
            315                 320                 325

act ggc tat aca aag aga ctg caa aaa cag gtc act ctg tcc cct ccc     1303
Thr Gly Tyr Thr Lys Arg Leu Gln Lys Gln Val Thr Leu Ser Pro Pro
        330                 335                 340

cca cta cct cca cca agt gca ctc att cag cag aac ctg cag cct cct     1351
Pro Leu Pro Pro Pro Ser Ala Leu Ile Gln Gln Asn Leu Gln Pro Pro
    345                 350                 355

atg aaa tct ccc aca tca aga caa cag gat cct ctg aag att cca gtg     1399
Met Lys Ser Pro Thr Ser Arg Gln Gln Asp Pro Leu Lys Ile Pro Val
360                 365                 370                 375

aca tcg tcc tca gct cac cca act ccc tct gta acc tca tta gct tca     1447
Thr Ser Ser Ser Ala His Pro Thr Pro Ser Val Thr Ser Leu Ala Ser
                380                 385                 390

aat cca tct tcc tcc gct cct tct gtg cct gga aac cca tct tct gcc     1495
Asn Pro Ser Ser Ser Ala Pro Ser Val Pro Gly Asn Pro Ser Ser Ala
            395                 400                 405

cca gct cca gta cct gat aca act gca aga gta tgt ata tca gtc cat     1543
Pro Ala Pro Val Pro Asp Thr Thr Ala Arg Val Cys Ile Ser Val His
        410                 415                 420

tca gaa aaa tca gat gga ccc ttt cgg gaa tca gaa aac aaa tta tta     1591
Ser Glu Lys Ser Asp Gly Pro Phe Arg Glu Ser Glu Asn Lys Leu Leu
    425                 430                 435

cca gct act gcc ctt aca tca gaa cat tca aag gaa gcc tct tca att     1639
Pro Ala Thr Ala Leu Thr Ser Glu His Ser Lys Glu Ala Ser Ser Ile
440                 445                 450                 455

gct gtt act gct cct atg gaa gaa aag cgt ggc cag gtg cca gtc ctt     1687
Ala Val Thr Ala Pro Met Glu Glu Lys Arg Gly Gln Val Pro Val Leu
                460                 465                 470

gaa act cca cct ttg ttg gga cag tca tta tta tac aaa cag ttt atc     1735
Glu Thr Pro Pro Leu Leu Gly Gln Ser Leu Leu Tyr Lys Gln Phe Ile
            475                 480                 485

cct aca act ggt cca gta aga ata aat gct gct cat cca ggt ggt ggt     1783
Pro Thr Thr Gly Pro Val Arg Ile Asn Ala Ala His Pro Gly Gly Gly
        490                 495                 500

caa cca gat tgg gaa cat tcc aac aag cat ggc ttg cct ttc tcc atc     1831
Gln Pro Asp Trp Glu His Ser Asn Lys His Gly Leu Pro Phe Ser Ile
    505                 510                 515

ttg ata tcc ctt gtg ttt ttt ggt ctg ggt gac tgt act gag gag ttt     1879
Leu Ile Ser Leu Val Phe Phe Gly Leu Gly Asp Cys Thr Glu Glu Phe
520                 525                 530                 535

gcc tct ttt gtc cct gga ttg tct cag atc tcc tgg tag                 1918
Ala Ser Phe Val Pro Gly Leu Ser Gln Ile Ser Trp
                540                 545
```

<210> SEQ ID NO 51
<211> LENGTH: 547
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 51

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```
Met Ser Cys Val His Tyr Lys Phe Ser Ser Lys Leu Ser Tyr Asn Thr
1               5                   10                  15

Ile Thr Phe Asp Gly Leu His Ile Ser Leu Phe Tyr Leu Lys Lys Gln
            20                  25                  30

Ile Met Gly Arg Glu Lys Leu Lys Thr Gly Asn Ser Asp Leu Gln Ile
        35                  40                  45

Ile Asn Ala Glu Thr Glu Glu Glu Tyr Thr Asp Asp Asn Ala Leu Ile
    50                  55                  60

Pro Lys Asn Ser Ser Val Ile Val Arg Arg Ile Pro Val Val Gly Val
65                  70                  75                  80

Lys Ser Lys Ser Lys Thr Tyr Gln Ile Ser His Thr Lys Ser Val Met
                85                  90                  95

Gly Thr Thr Arg Ala Val Asn Asp Ser Ser Ala Pro Met Ser Leu Ala
            100                 105                 110

Gln Leu Ile Glu Thr Ala Asn Leu Ala Glu Ala Asn Ala Ser Glu Glu
        115                 120                 125

Asp Lys Ile Lys Ala Met Met Ile Gln Ser Gly His Glu Tyr Asp Pro
    130                 135                 140

Ile Asn Tyr Met Lys Lys Thr Pro Val Gly Leu Pro Pro Pro Ser Tyr
145                 150                 155                 160

Thr Cys Phe Arg Cys Gly Lys Pro Gly His Tyr Thr Lys Asn Cys Pro
                165                 170                 175

Thr Ser Val Asn Lys Asp Phe Glu Ser Cys Pro Arg Ile Arg Lys Ser
            180                 185                 190

Thr Gly Ile Pro Arg Asn Phe Met Met Glu Val Lys Asp Pro Asn Met
        195                 200                 205

Lys Gly Ala Met Leu Thr Lys Thr Gly Gln Tyr Ala Ile Pro Thr Ile
    210                 215                 220

Asn Ala Glu Ala Tyr Ala Ile Gly Lys Lys Arg Lys Pro Pro Phe Leu
225                 230                 235                 240

Pro Gly Glu Pro Ser Ser Ser Ser Ser Glu Glu Val Gly Pro Val Pro
                245                 250                 255

Glu Glu Leu Leu Cys Leu Ile Cys Lys Asp Thr Met Thr Asp Ala Ala
            260                 265                 270

Ile Ile Pro Cys Cys Gly Asn Ser Tyr Cys Asp Glu Cys Ile Arg Thr
        275                 280                 285

Ala Leu Leu Glu Ser Asp Glu His Thr Cys Pro Thr Cys His Gln Asn
    290                 295                 300

Asp Val Ser Pro Asp Ala Leu Val Ala Asn Lys Val Leu Arg Gln Ala
305                 310                 315                 320

Val Asn Asn Phe Lys Asn Gln Thr Gly Tyr Thr Lys Arg Leu Gln Lys
                325                 330                 335

Gln Val Thr Leu Ser Pro Pro Pro Leu Pro Pro Pro Ser Ala Leu Ile
            340                 345                 350

Gln Gln Asn Leu Gln Pro Pro Met Lys Ser Pro Thr Ser Arg Gln Gln
        355                 360                 365

Asp Pro Leu Lys Ile Pro Val Thr Ser Ser Ser Ala His Pro Thr Pro
    370                 375                 380

Ser Val Thr Ser Leu Ala Ser Asn Pro Ser Ser Ser Ala Pro Ser Val
385                 390                 395                 400

Pro Gly Asn Pro Ser Ser Ala Pro Ala Pro Val Pro Asp Thr Thr Ala
                405                 410                 415

Arg Val Cys Ile Ser Val His Ser Glu Lys Ser Asp Gly Pro Phe Arg
            420                 425                 430
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Glu Ser Glu Asn Lys Leu Leu Pro Ala Thr Ala Leu Thr Ser Glu His
        435                 440                 445

Ser Lys Glu Ala Ser Ser Ile Ala Val Thr Ala Pro Met Glu Glu Lys
    450                 455                 460

Arg Gly Gln Val Pro Val Leu Glu Thr Pro Pro Leu Leu Gly Gln Ser
465                 470                 475                 480

Leu Leu Tyr Lys Gln Phe Ile Pro Thr Thr Gly Pro Val Arg Ile Asn
                485                 490                 495

Ala Ala His Pro Gly Gly Gly Gln Pro Asp Trp Glu His Ser Asn Lys
            500                 505                 510

His Gly Leu Pro Phe Ser Ile Leu Ile Ser Leu Val Phe Phe Gly Leu
        515                 520                 525

Gly Asp Cys Thr Glu Glu Phe Ala Ser Phe Val Pro Gly Leu Ser Gln
    530                 535                 540

Ile Ser Trp
545


<210> SEQ ID NO 52
<211> LENGTH: 3680
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (606)..(2558)

<400> SEQUENCE: 52

agatcagctt tttcatctga aagcaacgag tctatcggat ccttgaggtg ggaggcaaag     60

aacgcgatta ttttagtgat cctcgctggg agaggtacag attcgtgggt cagacggagg    120

gacaatggat tcctgggcct ggaggttcca gacattccct aatcatttac cctttccaaa    180

gcactggaac cacactgacc ctgataccta ctaattggtt attgaagggg gtgtgcaagt    240

ctcagcctgt tttcacttcc agccagtctc tttcccatcg cccaacgtgt gattattgtt    300

ctgcttcctg ggtagaagtc cctaacgagt cccctgttgg cctgggtgag tctcctcaac    360

aagcttcttt tctgagcagg aacacctttc taatgtggac attgcaggac aatcgctcgc    420

gaatcctaag tgcatgtgac cccaccttcc agcagcagag gacgtttctc ctcgctccag    480

agtgcttgga atatcttggt ggcaccttct gttaccagtg acaacctgtt gacactaaga    540

ggtctggaca ggatttcccg tcaccgcagc cataccacct attacatctc gattttctgt    600

gactt atg cgc tcc ggt ctc tgc acg cct gca gag gca ttg gag atg cct    650
      Met Arg Ser Gly Leu Cys Thr Pro Ala Glu Ala Leu Glu Met Pro
      1               5                  10                  15

tct agc aca gag gcg gcg acc gat gaa tgt gac gat gcg gag ctc cgg      698
Ser Ser Thr Glu Ala Ala Thr Asp Glu Cys Asp Asp Ala Glu Leu Arg
               20                  25                  30

tgc cgg gta gcc gtg gag gag ctg agt cct gga ggg caa cct cgc aag      746
Cys Arg Val Ala Val Glu Glu Leu Ser Pro Gly Gly Gln Pro Arg Lys
            35                  40                  45

cgc cag gcc ctg cgc gcc gca gag ctg agc cta ggt cga aac gaa cga      794
Arg Gln Ala Leu Arg Ala Ala Glu Leu Ser Leu Gly Arg Asn Glu Arg
        50                  55                  60

cgt gag tta atg ctg cga ctg cag gca ccg gga ccc acg ggg cgg cca      842
Arg Glu Leu Met Leu Arg Leu Gln Ala Pro Gly Pro Thr Gly Arg Pro
    65                  70                  75

cgc tgt ttc ccg cta cgc gcc gtg cgc ctc ttc acc cgc ttc gct gcg      890
Arg Cys Phe Pro Leu Arg Ala Val Arg Leu Phe Thr Arg Phe Ala Ala
80                  85                  90                  95
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act ggg cgc agc acg ttg cgg ctc ccc acc gat gga gtc cct gga gct      938
Thr Gly Arg Ser Thr Leu Arg Leu Pro Thr Asp Gly Val Pro Gly Ala
                100                 105                 110

ggc tca gtg caa ctg ctc ctc tcc gac tgt ccc ccg gag cgc ttg cgc      986
Gly Ser Val Gln Leu Leu Leu Ser Asp Cys Pro Pro Glu Arg Leu Arg
            115                 120                 125

cgc ttc ctg cgc acg ctg cgc ctg aag ctg gcg gtt gcc cct ggg ccg     1034
Arg Phe Leu Arg Thr Leu Arg Leu Lys Leu Ala Val Ala Pro Gly Pro
        130                 135                 140

gga ccc gcc tct gcc cgc gca cag ttg ctc ggc ccg cgg ccc cga gac     1082
Gly Pro Ala Ser Ala Arg Ala Gln Leu Leu Gly Pro Arg Pro Arg Asp
    145                 150                 155

ttt gtc acc atc agt cca gtg cag cca gag gaa ctg cag cgt gct gca     1130
Phe Val Thr Ile Ser Pro Val Gln Pro Glu Glu Leu Gln Arg Ala Ala
160                 165                 170                 175

gcc acc aag gct cca gat tct gcg ctg gaa aag cgg cca atg gaa tcc     1178
Ala Thr Lys Ala Pro Asp Ser Ala Leu Glu Lys Arg Pro Met Glu Ser
                180                 185                 190

cag act agt acg gaa gct cca agg tgg ccc ctg cct gtg aag aag ctg     1226
Gln Thr Ser Thr Glu Ala Pro Arg Trp Pro Leu Pro Val Lys Lys Leu
            195                 200                 205

cgc atg ccc tcc acc aaa ccg aag ctt tct gaa gag cag gcc gct gtg     1274
Arg Met Pro Ser Thr Lys Pro Lys Leu Ser Glu Glu Gln Ala Ala Val
        210                 215                 220

ctg agg atg gtt ctg aaa ggc cag agc att ttc ttc act ggg agc gca     1322
Leu Arg Met Val Leu Lys Gly Gln Ser Ile Phe Phe Thr Gly Ser Ala
    225                 230                 235

ggg aca gga aag tcc tac ctg ctg aaa cat atc ctg ggt tcc ctg ccc     1370
Gly Thr Gly Lys Ser Tyr Leu Leu Lys His Ile Leu Gly Ser Leu Pro
240                 245                 250                 255

cct act ggt act gtg gcc act gcc agc act ggg gtg gca gcc tgc cac     1418
Pro Thr Gly Thr Val Ala Thr Ala Ser Thr Gly Val Ala Ala Cys His
                260                 265                 270

att ggg ggc acc acc ctt cat gcc ttt gca ggc atc ggc tca ggc cag     1466
Ile Gly Gly Thr Thr Leu His Ala Phe Ala Gly Ile Gly Ser Gly Gln
            275                 280                 285

gct ccc ctg gcc cag tgc atg gcc ctg gcc aat cgg cca ggt gtg cgg     1514
Ala Pro Leu Ala Gln Cys Met Ala Leu Ala Asn Arg Pro Gly Val Arg
        290                 295                 300

cag ggc tgg ctg aac tgc caa cgt ttg gtc att gac gag atc tcc atg     1562
Gln Gly Trp Leu Asn Cys Gln Arg Leu Val Ile Asp Glu Ile Ser Met
    305                 310                 315

gtg gag gca gac ttc ttt gac aag ttg gaa gct gtg gcc aga gct gtc     1610
Val Glu Ala Asp Phe Phe Asp Lys Leu Glu Ala Val Ala Arg Ala Val
320                 325                 330                 335

cgg caa cag aag aag cca ttt gga ggg atc cag ctc atc atc tgt ggg     1658
Arg Gln Gln Lys Lys Pro Phe Gly Gly Ile Gln Leu Ile Ile Cys Gly
                340                 345                 350

gac ttc cta cag ttg cca cca gtg acc aaa ggc tcc cag cag cct cag     1706
Asp Phe Leu Gln Leu Pro Pro Val Thr Lys Gly Ser Gln Gln Pro Gln
            355                 360                 365

ttc tgc ttt cag gcc aag agc tgg agg agg tgt gtg cct gtg att ctg     1754
Phe Cys Phe Gln Ala Lys Ser Trp Arg Arg Cys Val Pro Val Ile Leu
        370                 375                 380

gag ctg act gag gtg tgg agg caa gca gac cag acc ttc atc tct cta     1802
Glu Leu Thr Glu Val Trp Arg Gln Ala Asp Gln Thr Phe Ile Ser Leu
    385                 390                 395

ctg cag gct gtg agg tta ggc aga tgt tca gat gaa gta acc cgc cag     1850
Leu Gln Ala Val Arg Leu Gly Arg Cys Ser Asp Glu Val Thr Arg Gln
400                 405                 410                 415
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ctc agg gcc aca gct gcc cat aag gtg gga cga gat gga att gta gcc     1898
Leu Arg Ala Thr Ala Ala His Lys Val Gly Arg Asp Gly Ile Val Ala
                420                 425                 430

acg aga cta tgt acc cat cag gat gat gtg gcc ctg acc aac gag aag     1946
Thr Arg Leu Cys Thr His Gln Asp Asp Val Ala Leu Thr Asn Glu Lys
            435                 440                 445

tgg ctg aag gca ctg cca ggt gat gta cac agc ttt gag gct ata gac     1994
Trp Leu Lys Ala Leu Pro Gly Asp Val His Ser Phe Glu Ala Ile Asp
        450                 455                 460

agt gac cct gag cta agc cgg acc ctg gat gct cag tgc cct gtt agc     2042
Ser Asp Pro Glu Leu Ser Arg Thr Leu Asp Ala Gln Cys Pro Val Ser
    465                 470                 475

cgt gtc ctt cag tta aag ctg ggg gct cag gtc atg ctg gtg aag aac     2090
Arg Val Leu Gln Leu Lys Leu Gly Ala Gln Val Met Leu Val Lys Asn
480                 485                 490                 495

ttg gca gtg tct cgg ggc ctg gtg aac ggt gcc cga ggg gtg gta gtt     2138
Leu Ala Val Ser Arg Gly Leu Val Asn Gly Ala Arg Gly Val Val Val
                500                 505                 510

ggg ttt gag tcc gaa ggg aga ggg ctc ccc cgg gta cgg ttc ctg tgt     2186
Gly Phe Glu Ser Glu Gly Arg Gly Leu Pro Arg Val Arg Phe Leu Cys
            515                 520                 525

ggt atc act gag gtc atc cgc act gac cgc tgg aca gta cag gtc act     2234
Gly Ile Thr Glu Val Ile Arg Thr Asp Arg Trp Thr Val Gln Val Thr
        530                 535                 540

ggg gga cag tac ctc agc cgg cag cag ctt ccc cta cag ctg gcc tgg     2282
Gly Gly Gln Tyr Leu Ser Arg Gln Gln Leu Pro Leu Gln Leu Ala Trp
    545                 550                 555

gcc ata tcc atc cac aaa agc cag ggc atg tct ctg gac tgt gtg gag     2330
Ala Ile Ser Ile His Lys Ser Gln Gly Met Ser Leu Asp Cys Val Glu
560                 565                 570                 575

atc tct ctg ggc cgt gtg ttt gcc agt ggt caa gcc tat gtg gcc ctc     2378
Ile Ser Leu Gly Arg Val Phe Ala Ser Gly Gln Ala Tyr Val Ala Leu
                580                 585                 590

tcc cgg gcc cgt agc ctc cag ggt ctt cgt gtg ctg gac ttt gac ccc     2426
Ser Arg Ala Arg Ser Leu Gln Gly Leu Arg Val Leu Asp Phe Asp Pro
            595                 600                 605

acg gtg gtt cga tgt gac tcc cga gtg ctg cat ttc tat gcc acc ctg     2474
Thr Val Val Arg Cys Asp Ser Arg Val Leu His Phe Tyr Ala Thr Leu
        610                 615                 620

cgg cag ggc agg ggc ctc agt ctg gag tcc caa gac gat gag gag gca     2522
Arg Gln Gly Arg Gly Leu Ser Leu Glu Ser Gln Asp Asp Glu Glu Ala
    625                 630                 635

aac tca gat ctg gag aac atg gac cca aac ctc tga cctcagctga          2568
Asn Ser Asp Leu Glu Asn Met Asp Pro Asn Leu
640                 645                 650

aagagaagac aaacttttag ctttttttcc tgggtcaagg ccctaggaat taactgggga   2628

gaggcctgtg tttcttccct tattcagcct ctggtagggt taagggacac agtttcccat   2688

ctacttaact agcattgcct cagtttcacc tatttccccg gggaaatgac ttccagggtt   2748

caaagctaga aatggtgatg gttaccagag gacaaagctc tctaccaagg gtggaacaca   2808

cagccacaga gttctttgca ggctggagag gcagtgcggg caggggctgc attcagcagc   2868

agcagcagta ggagcagcct gtcttattac accgcatgta tttattttgt gtgcttgtgc   2928

acgcacagca tattgtacat gtgaaggtca gaggacaact cgaggaagtt ggttttctct   2988

ttccccaagt gtgttctggg ggttaaattc aggtcacagg gcttggtagc aggcacttat   3048

acccgatgag caatcttgct accagggtcg gttctaattt tctttgtgtt attataacaa   3108

aatatataag gctgagtact ttatgaaaaa aatgatttat ttttaattaa tatatgctca   3168
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cagttctaga agatgaagaa cacggaacca gcctcgtctc agctttgctg gggtttgacg   3228

gtagcagcaa ccgcctggcg gggacacttg caggcaggat catgagagac aggcacagag   3288

gatggtgatg ctggggaaga gtattaatcc gtccatgagg acaggacccc cttgctttag   3348

ttacctccca tgcaatccat ctctgaaagg ttacatcatc ttaacactgc tacgctaggg   3408

actaagcttc cagtacataa acctataagg gaaaccatcc aaactatggc aggagcctag   3468

aggggattca ggccagacac aagcccaaga tagaagttta attaccttca cagctgtgct   3528

cagcctagca cagccccaag taaacatcat tcagagcccg actgagaaca gacgctgcaa   3588

aatgtgctgg gtttagggga gaggccgtgt ttaggatacg gagatgtatg ttctcctttg   3648

tatttattta agccaaataa aactgtgaac cg                                 3680


<210> SEQ ID NO 53
<211> LENGTH: 650
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 53

Met Arg Ser Gly Leu Cys Thr Pro Ala Glu Ala Leu Glu Met Pro Ser
1               5                   10                  15

Ser Thr Glu Ala Ala Thr Asp Glu Cys Asp Asp Ala Glu Leu Arg Cys
            20                  25                  30

Arg Val Ala Val Glu Glu Leu Ser Pro Gly Gly Gln Pro Arg Lys Arg
        35                  40                  45

Gln Ala Leu Arg Ala Ala Glu Leu Ser Leu Gly Arg Asn Glu Arg Arg
    50                  55                  60

Glu Leu Met Leu Arg Leu Gln Ala Pro Gly Pro Thr Gly Arg Pro Arg
65                  70                  75                  80

Cys Phe Pro Leu Arg Ala Val Arg Leu Phe Thr Arg Phe Ala Ala Thr
                85                  90                  95

Gly Arg Ser Thr Leu Arg Leu Pro Thr Asp Gly Val Pro Gly Ala Gly
            100                 105                 110

Ser Val Gln Leu Leu Leu Ser Asp Cys Pro Pro Glu Arg Leu Arg Arg
        115                 120                 125

Phe Leu Arg Thr Leu Arg Leu Lys Leu Ala Val Ala Pro Gly Pro Gly
    130                 135                 140

Pro Ala Ser Ala Arg Ala Gln Leu Leu Gly Pro Arg Pro Arg Asp Phe
145                 150                 155                 160

Val Thr Ile Ser Pro Val Gln Pro Glu Glu Leu Gln Arg Ala Ala Ala
                165                 170                 175

Thr Lys Ala Pro Asp Ser Ala Leu Glu Lys Arg Pro Met Glu Ser Gln
            180                 185                 190

Thr Ser Thr Glu Ala Pro Arg Trp Pro Leu Pro Val Lys Lys Leu Arg
        195                 200                 205

Met Pro Ser Thr Lys Pro Lys Leu Ser Glu Glu Gln Ala Ala Val Leu
    210                 215                 220

Arg Met Val Leu Lys Gly Gln Ser Ile Phe Phe Thr Gly Ser Ala Gly
225                 230                 235                 240

Thr Gly Lys Ser Tyr Leu Leu Lys His Ile Leu Gly Ser Leu Pro Pro
                245                 250                 255

Thr Gly Thr Val Ala Thr Ala Ser Thr Gly Val Ala Ala Cys His Ile
            260                 265                 270

Gly Gly Thr Thr Leu His Ala Phe Ala Gly Ile Gly Ser Gly Gln Ala
        275                 280                 285
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Pro Leu Ala Gln Cys Met Ala Leu Ala Asn Arg Pro Gly Val Arg Gln
    290                 295                 300

Gly Trp Leu Asn Cys Gln Arg Leu Val Ile Asp Glu Ile Ser Met Val
305                 310                 315                 320

Glu Ala Asp Phe Phe Asp Lys Leu Glu Ala Val Ala Arg Ala Val Arg
                325                 330                 335

Gln Gln Lys Lys Pro Phe Gly Gly Ile Gln Leu Ile Ile Cys Gly Asp
            340                 345                 350

Phe Leu Gln Leu Pro Pro Val Thr Lys Gly Ser Gln Gln Pro Gln Phe
        355                 360                 365

Cys Phe Gln Ala Lys Ser Trp Arg Arg Cys Val Pro Val Ile Leu Glu
    370                 375                 380

Leu Thr Glu Val Trp Arg Gln Ala Asp Gln Thr Phe Ile Ser Leu Leu
385                 390                 395                 400

Gln Ala Val Arg Leu Gly Arg Cys Ser Asp Glu Val Thr Arg Gln Leu
                405                 410                 415

Arg Ala Thr Ala Ala His Lys Val Gly Arg Asp Gly Ile Val Ala Thr
            420                 425                 430

Arg Leu Cys Thr His Gln Asp Asp Val Ala Leu Thr Asn Glu Lys Trp
        435                 440                 445

Leu Lys Ala Leu Pro Gly Asp Val His Ser Phe Glu Ala Ile Asp Ser
    450                 455                 460

Asp Pro Glu Leu Ser Arg Thr Leu Asp Ala Gln Cys Pro Val Ser Arg
465                 470                 475                 480

Val Leu Gln Leu Lys Leu Gly Ala Gln Val Met Leu Val Lys Asn Leu
                485                 490                 495

Ala Val Ser Arg Gly Leu Val Asn Gly Ala Arg Gly Val Val Val Gly
            500                 505                 510

Phe Glu Ser Glu Gly Arg Gly Leu Pro Arg Val Arg Phe Leu Cys Gly
        515                 520                 525

Ile Thr Glu Val Ile Arg Thr Asp Arg Trp Thr Val Gln Val Thr Gly
    530                 535                 540

Gly Gln Tyr Leu Ser Arg Gln Gln Leu Pro Leu Gln Leu Ala Trp Ala
545                 550                 555                 560

Ile Ser Ile His Lys Ser Gln Gly Met Ser Leu Asp Cys Val Glu Ile
                565                 570                 575

Ser Leu Gly Arg Val Phe Ala Ser Gly Gln Ala Tyr Val Ala Leu Ser
            580                 585                 590

Arg Ala Arg Ser Leu Gln Gly Leu Arg Val Leu Asp Phe Asp Pro Thr
        595                 600                 605

Val Val Arg Cys Asp Ser Arg Val Leu His Phe Tyr Ala Thr Leu Arg
    610                 615                 620

Gln Gly Arg Gly Leu Ser Leu Glu Ser Gln Asp Asp Glu Glu Ala Asn
625                 630                 635                 640

Ser Asp Leu Glu Asn Met Asp Pro Asn Leu
                645                 650


<210> SEQ ID NO 54
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 54

gtagcgatat gaggagatt                                                19
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<210> SEQ ID NO 55
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 55

gaccaacaat ttagagttt 19

<210> SEQ ID NO 56
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 56

caccaagtgc tcagctaaa 19

<210> SEQ ID NO 57
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 57

gctgcaaagt ctctggaag 19

<210> SEQ ID NO 58
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 58

ccagtggtag cgatatgagg agatt 25

<210> SEQ ID NO 59
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic oligonucleotide

<400> SEQUENCE: 59

gagtgaattg ctttgtgtc 19

<210> SEQ ID NO 60
<211> LENGTH: 1848
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (45)..(45)
<223> OTHER INFORMATION: n = C or T
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (49)..(49)
<223> OTHER INFORMATION: n = C or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (54)..(54)
<223> OTHER INFORMATION: n = C or A
<220> FEATURE:
<221> NAME/KEY: misc_feature

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<222> LOCATION: (55)..(55)
<223> OTHER INFORMATION: n = A or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (69)..(69)
<223> OTHER INFORMATION: n = A or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (81)..(81)
<223> OTHER INFORMATION: n = T or C
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (96)..(96)
<223> OTHER INFORMATION: n = T or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (139)..(139)
<223> OTHER INFORMATION: n = A or G
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (159)..(159)
<223> OTHER INFORMATION: n = T or C
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (166)..(166)
<223> OTHER INFORMATION: n = G or A
<220> FEATURE:
<221> NAME/KEY: misc_feature
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The invention claimed is:

1. An in vitro method of identifying a subpopulation of cultured human or mouse embryonic stem (ES) cells expressing Zscan4, comprising:

(a) transfecting a population of mouse or human ES cells with an expression vector comprising a Zscan4c promoter operably linked to a nucleotide sequence encoding a reporter, wherein the Zscan4c promoter is selected from group consisting of the nucleic acid sequence as set forth in nucleotides (i) 1-2540 of SEQ ID NO: 28, (ii) 1-2643 of SEQ ID NO: 28, (iii) 1-3250 of SEQ ID NO: 28 and (iv) 1-3347 of SEQ ID NO: 28; and

(b) identifying a subpopulation of cells that expresses the reporter gene indicating Zscan-4 is expressed in the subpopulation of stem cells.

2. The method of claim **1**, wherein the expression vector consists of the nucleotide sequence as set forth in SEQ ID NO: 28.

3. The method of claim **1**, wherein the reporter gene encodes a marker, enzyme, or fluorescent protein.

4. The method of claim **1**, wherein the expression vector is a viral vector.

5. The method of claim **1**, wherein the expression vector is a plasmid vector.

6. The method of claim **1**, wherein the population of embryonic stem cells are mouse embryonic stem cells.

7. The method of claim **1**, wherein the population of embryonic stem cells are human embryonic stem cells.

* * * * *