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(54) **ETS2 AND MESP1 GENERATE CARDIAC PROGENITORS FROM FIBROBLASTS**

(71) Applicants: **University of Houston**, Houston, TX (US); **Texas Heart Institute**, Houston, TX (US); **The Texas A&M University System**, College Station, TX (US)

(72) Inventors: **Robert J. Schwartz**, Houston, TX (US); **Vladimir N. Potaman**, Houston, TX (US); **Jose Francisco Islas**, Houston, TX (US)

(73) Assignees: **University of Houston**, Houston, TX (US); **Texas Heart Institute**, Houston, TX (US); **The Texas A&M University System**, College Station, TX (US)

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(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Jennifer Dunston

Assistant Examiner — Addison D Ault

(74) Attorney, Agent, or Firm — Jackson Walker L.L.P.

(57) **ABSTRACT**

A method for modulating cell differentiation capabilities using heterologous gene expression. Some embodiments of the invention relate to a method for inducing a cardiac progenitor cell by delivering a reprogramming factor to the cell, wherein the reprogramming factor comprises ETS2 or a combination of ETS2 and Mesp1.

10 Claims, 13 Drawing Sheets

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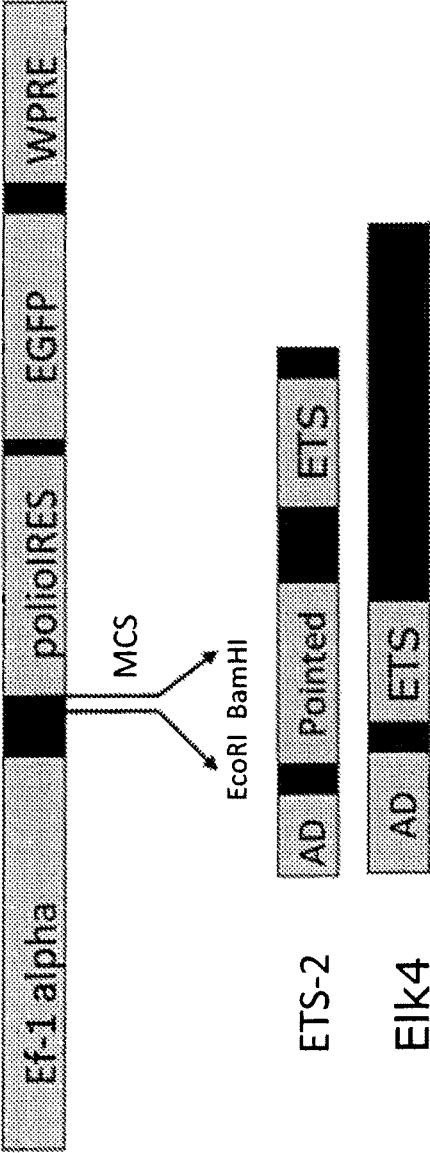


FIG. 1

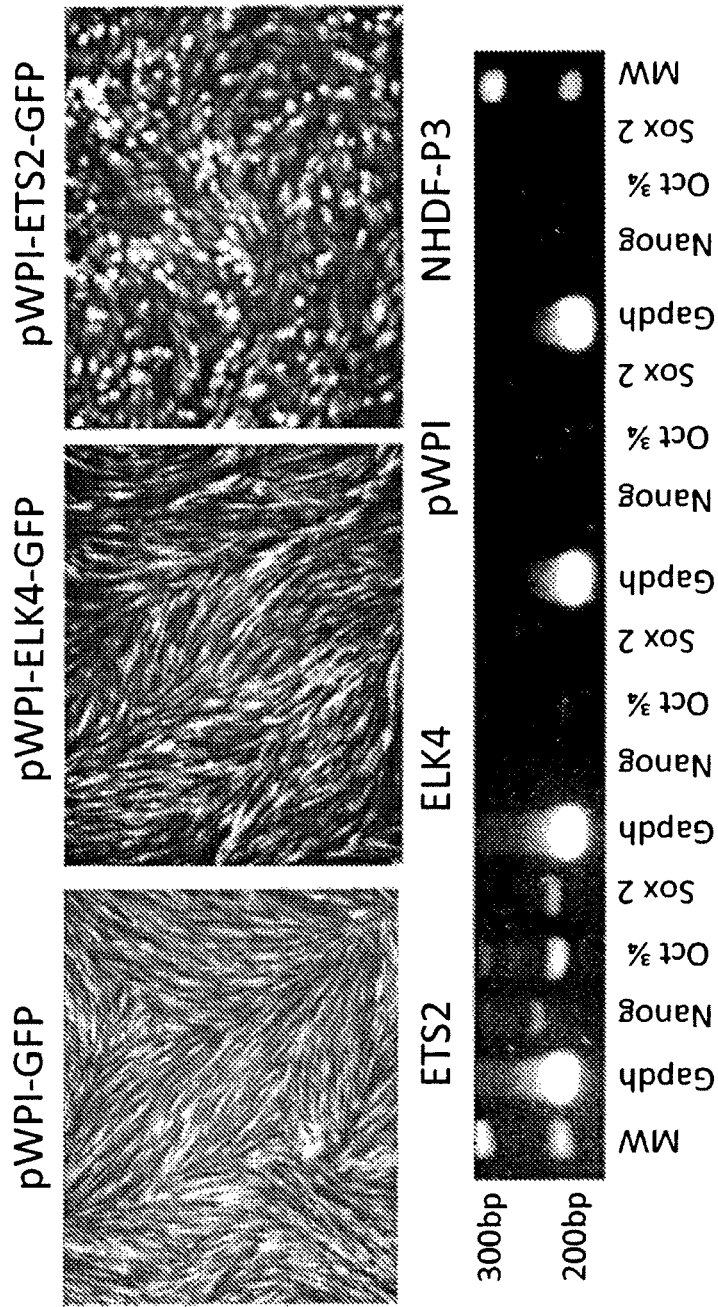


FIG. 2

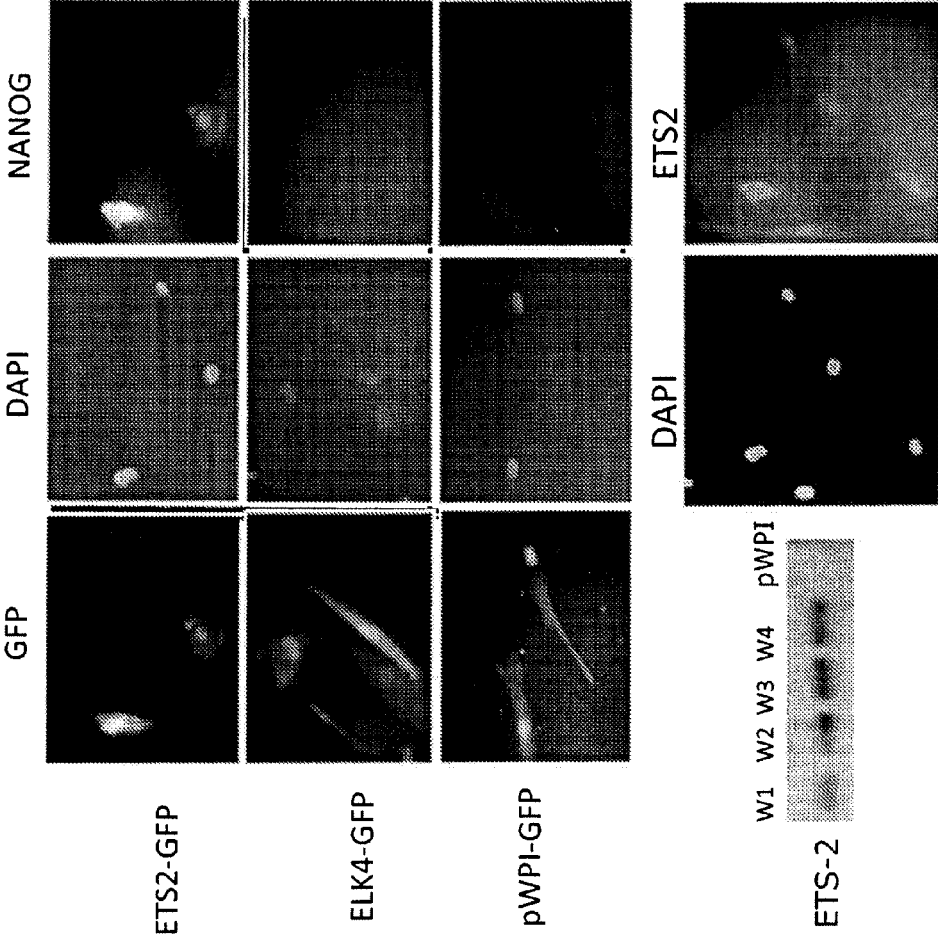


FIG. 3

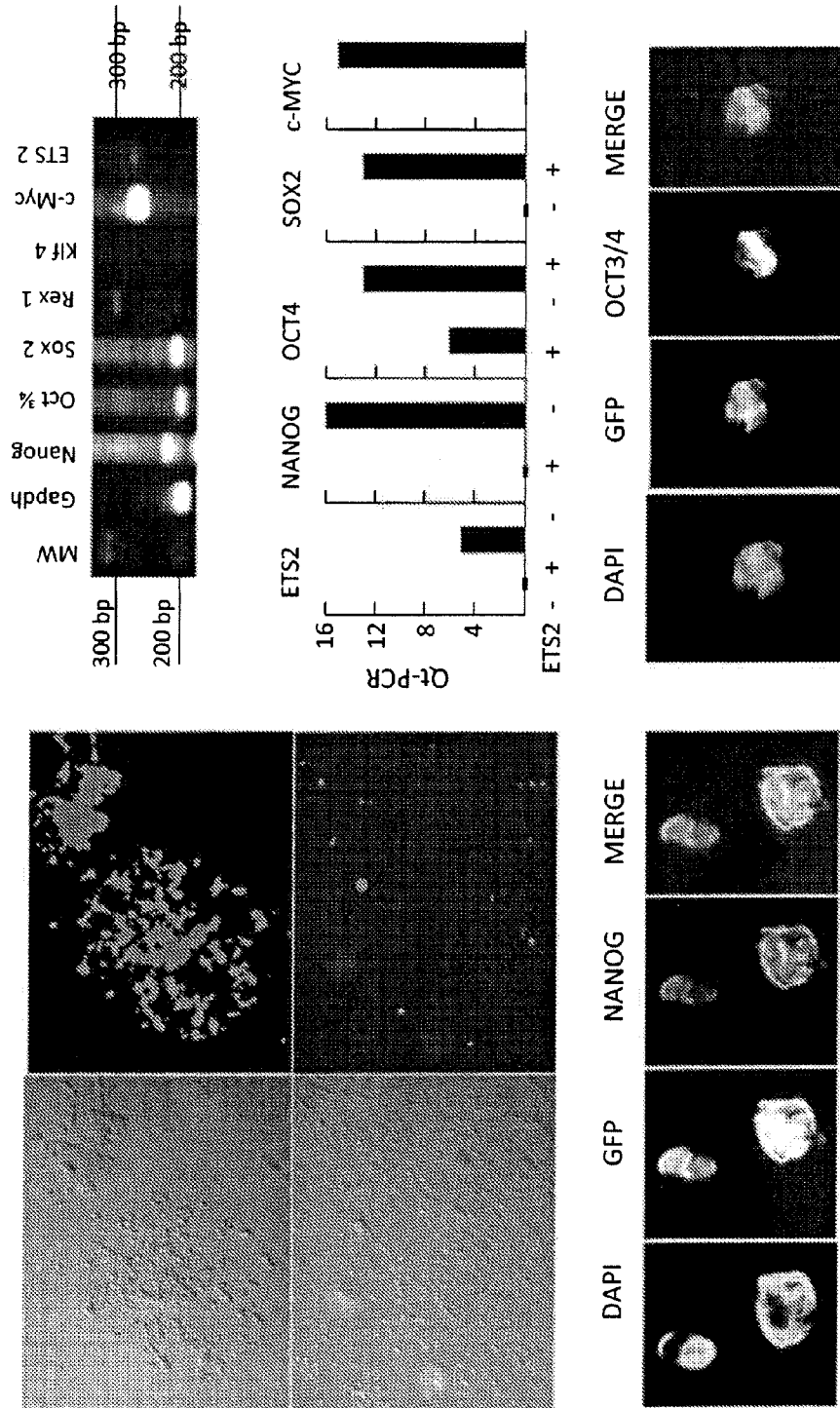


FIG. 4

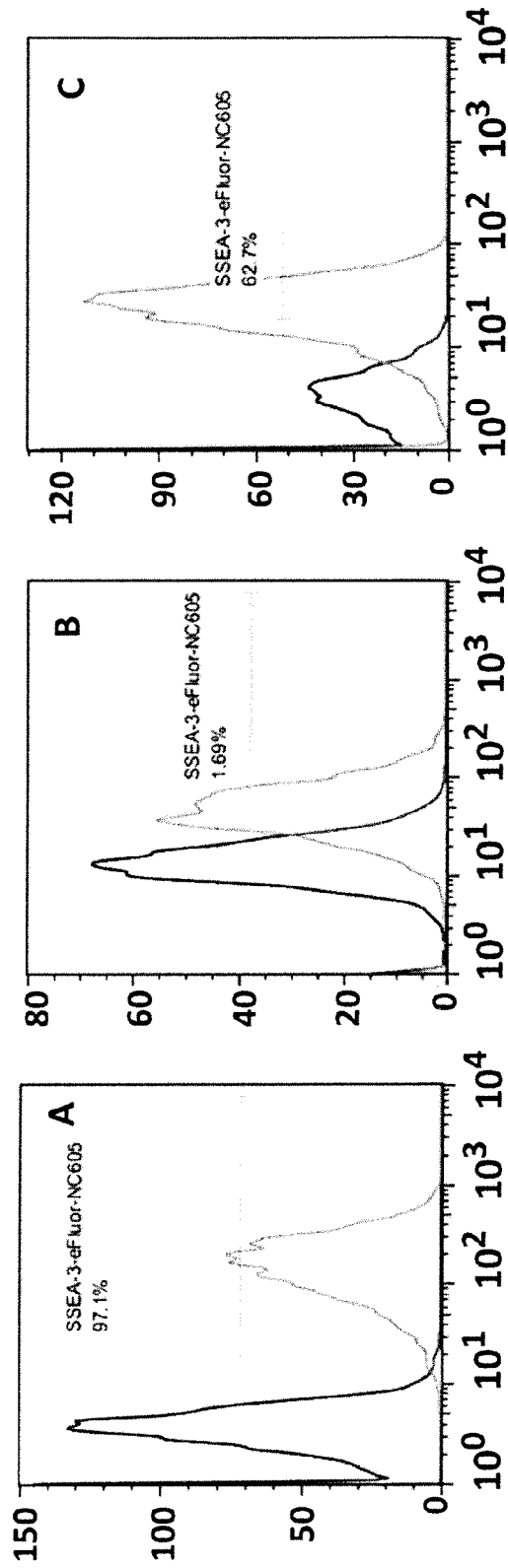


FIG. 5

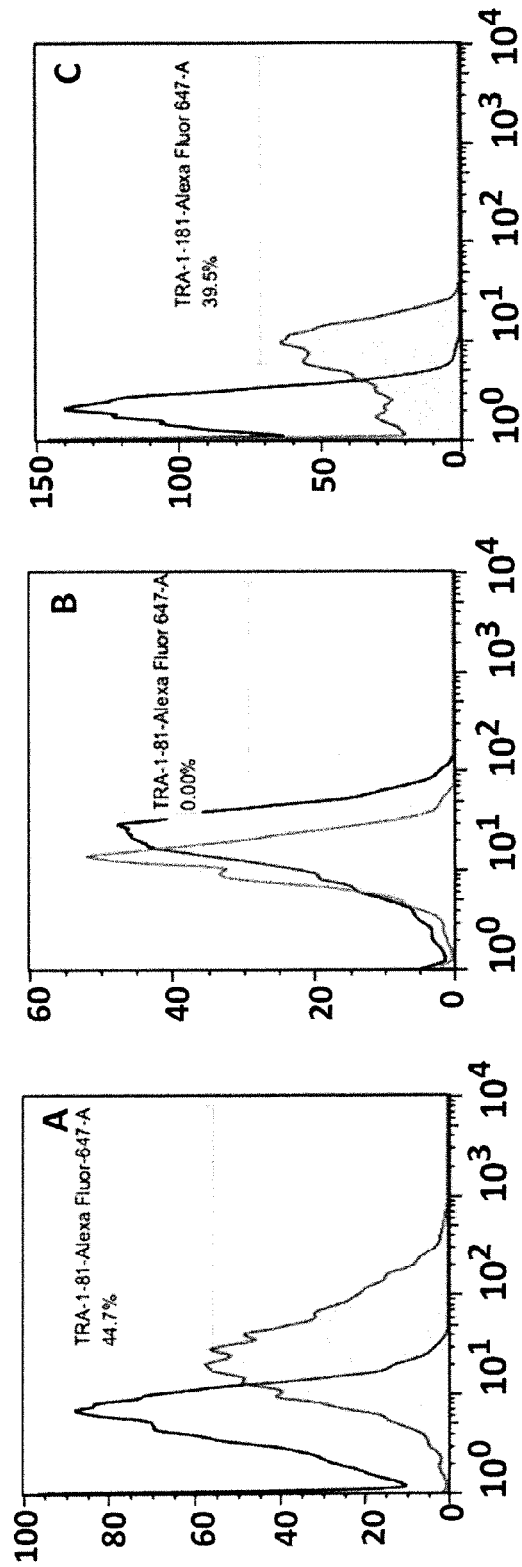


FIG. 6

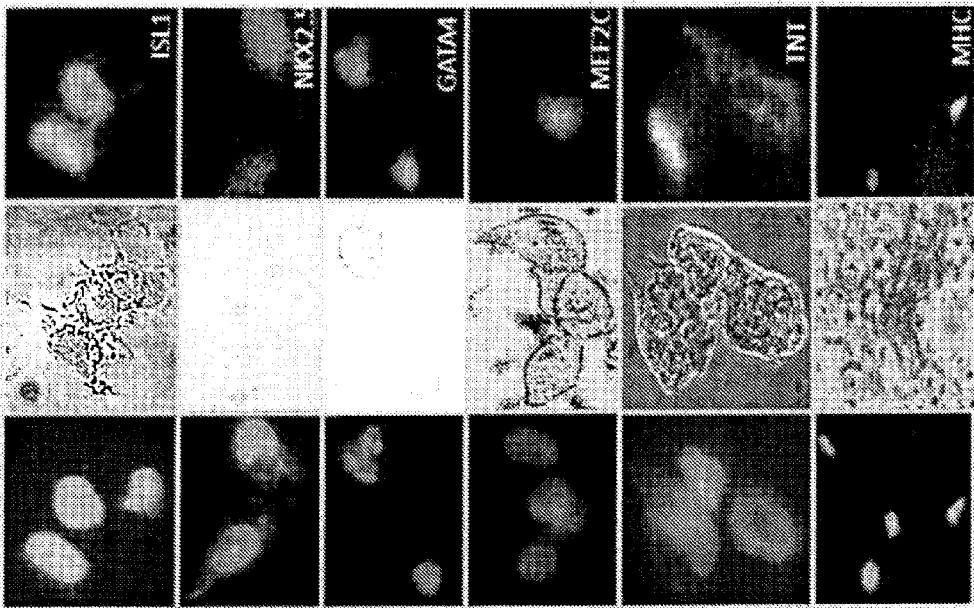


FIG. 7

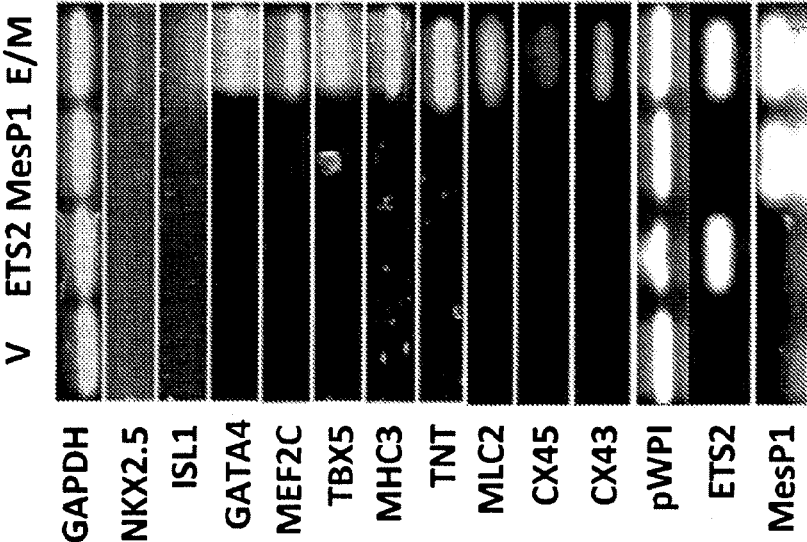


FIG. 8

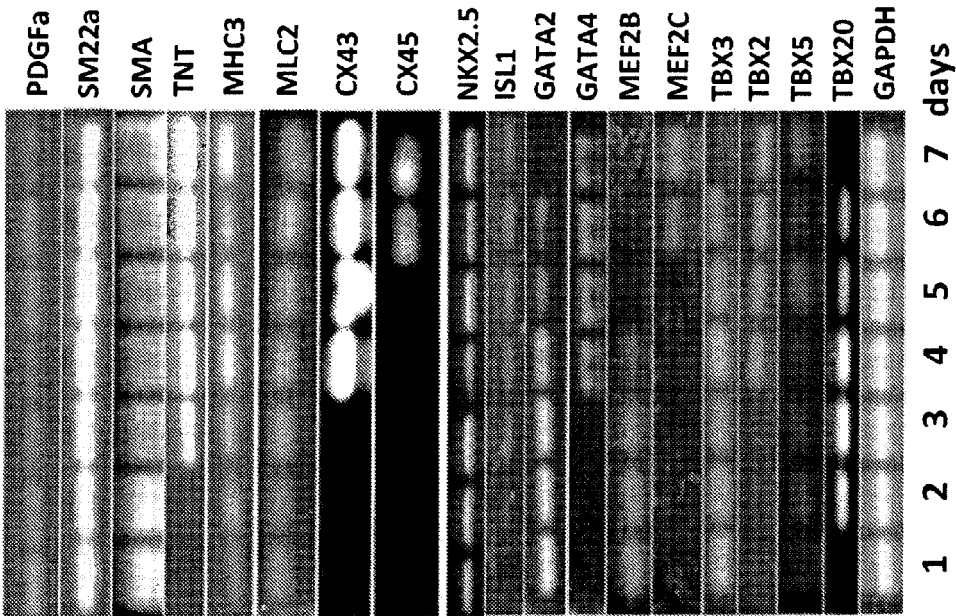


FIG. 9

ETS2 and Mesp1 induced de novo cardiac progenitor program gene expression:
Red-Tomato fluorescence staining from the reporter construct NKX2.5 Red-Tomato

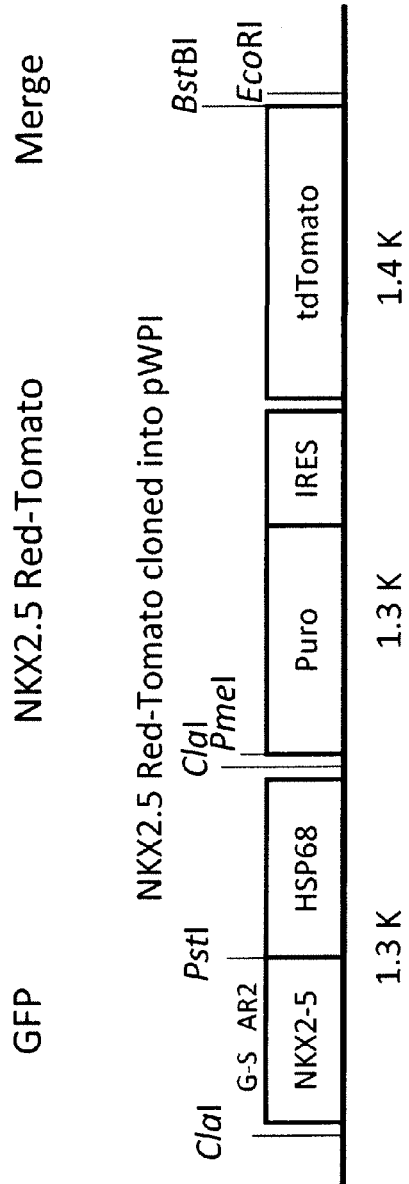
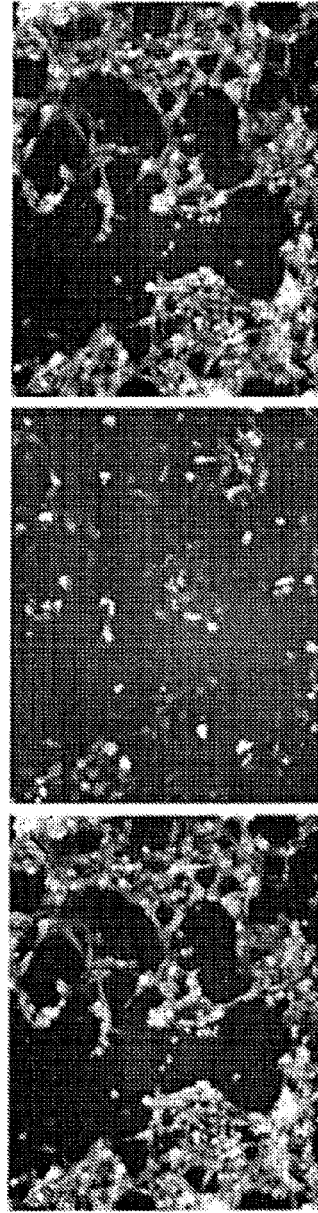


FIG. 10

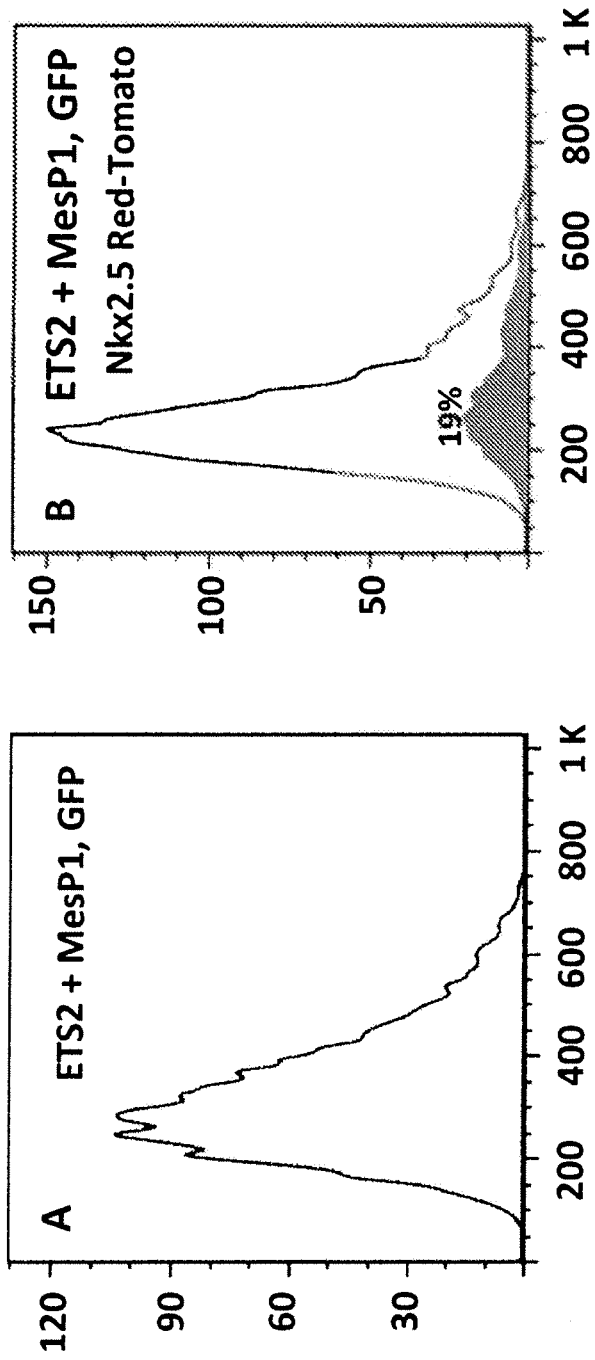


FIG. 11

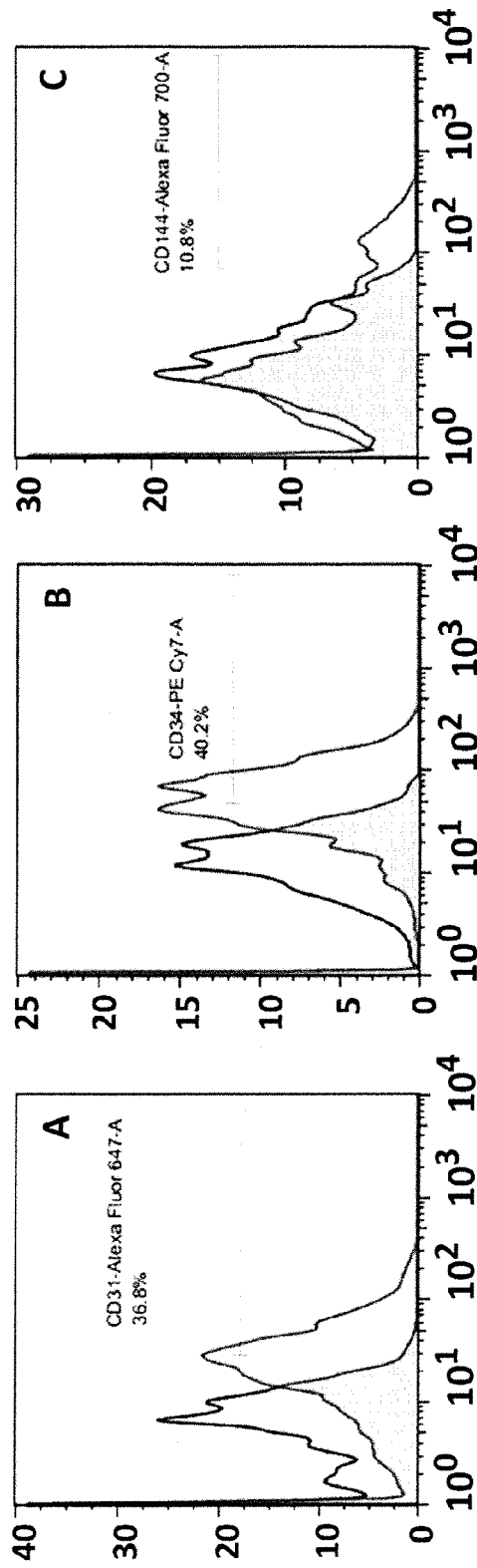


FIG. 12

ETS-2/Mesp1 Aggregates
9 Days Post Puromycin Selection

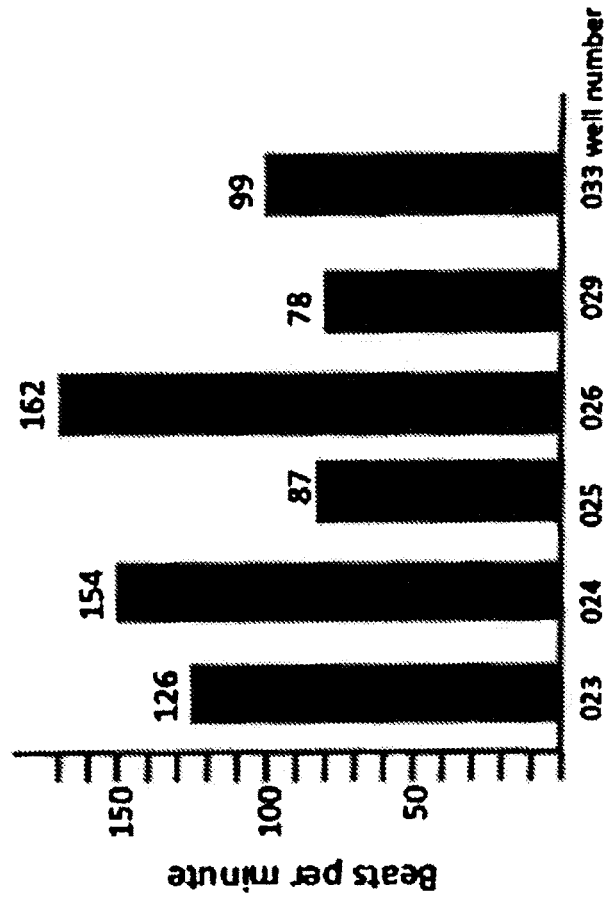


FIG. 13

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ETS2 AND MESP1 GENERATE CARDIAC PROGENITORS FROM FIBROBLASTS

This application claims priority to U.S. Provisional Patent Application Ser. No. 61/339,509, filed on Mar. 5, 2010, entitled ETS2 AND MESP1 GENERATE CARDIAC PROGENITORS FROM FIBROBLASTS, the entire content of which is hereby incorporated by reference.

FIELD OF THE INVENTION

One aspect of the present invention relates generally to the field of cell differentiation, and more specifically to a strategy for cardiovascular tissue regeneration via the isolation, renewal, and directed differentiation, of fibroblasts into specific mature cardiac, pacemaker, smooth muscle, and endothelial cell types.

BACKGROUND

Damage to mammalian heart tissue frequently results in the loss of large numbers of cardiac cells, including mature cardiac cells, pacemaker cells, smooth muscle, and endothelial cells. Although there is some indication that cardiac cells can be regenerated in humans (Bergmann et al., 2009), the mechanism is not well understood and the process does not appear to proceed rapidly enough to repair common types of cardiac damage such as ischemia, infarction, trauma, or injury due to toxins or viral infections. Therefore, a central goal of experimental cardiac medicine has been the development of a means for regenerating cardiac cells which have been lost due to cardiac damage. Studies of the mechanisms behind the embryonic cardiogenesis have been conducted, with the aim of replicating cardiogenesis in vitro or in vivo for the purposes of regenerating damaged tissue.

Recent research has identified multipotent (Isl1+) cardiovascular progenitor (MICP) cells, which are capable of differentiating to form mature cardiac tissue. MICP cells derived from embryonic stem (ES) cells which can give rise to endothelial, cardiac, and smooth muscle cells, have been isolated (Moretti et al., 2006). Genetic studies have shown that these MICP cells express Isl1, Nkx2.5 and Flk1.

Model systems for investigating cardiogenesis include the ascidian *Ciona intestinalis* (Beh et al., 2007). Lineage studies have shown that the adult *Ciona* heart is derived from two founder cells that express Ci-Mesp, a basic helix-loop-helix (bHLH) transcription factor, and also Ci-Ets1/2 (Imai et al., 2004; Satou et al., 2004). In addition, ascidian orthologs of the conserved heart specification genes NK4 (tinman Nkx2.5), GATAa (pannier/GATA4/5/6), Hand and Hand-like (Imai et al., 2003; Davidson, 2007; Davidson and Levine, 2003; Satou et al., 2004) are expressed. Ci-Mesp-knockdown embryos did not develop heart primordia, and target inhibition of Ets1/2 activity also blocked heart specification and the expansion of the heart field. Similarly, murine homologues of Ci-Mesp, Mesp1 and Mesp2 are expressed in the early mesoderm fated to become cranio-cardiac mesoderm (Saga et al., 2000). Only the Mesp1/Mesp2 double-knockout mouse lacked any cardiac mesoderm (Saga et al., 1999; Kitajima et al., 2000), indicating a role for these genes in directing the appearance of cardiac progenitors in higher vertebrates. Redundancies of Mesp genes have made further study in embryos a daunting task.

What is needed in the art is a method of inducing cardiogenesis for the purpose of regenerating cardiac cells for the use in the treatment of damaged cardiac tissue. Reprogramming of human somatic cells into pluripotent cells by a limited

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number of transcriptional factors important for maintaining self renewal and pluripotency has been reported by Yamanaka's, Thomson's and Daley's groups (Takahashi et al., 2007; Yu et al., 2007; Park et al., 2008). One aspect of the present invention provides a means of reprogramming the somatic cells and directed differentiation into cardiac progenitor cells. Therefore, one embodiment of this application provides a way to test a unique regulatory paradigm that ETS2 and Mesp1 are transformative, and unlike NKX2.5 and ISL1, convert non-embryonic normal human dermal fibroblasts (NHDFs) into primary cardiac progenitors. Another aspect of the present application was to elucidate the role of Mesp1 in the regulatory hierarchy directing the appearance of cardiac progenitors.

SUMMARY

One embodiment of the present invention relates to the modulation of cell differentiation capabilities using heterologous gene expression. Some embodiments of the invention relate to a method for inducing a cardiac progenitor cell by delivering a reprogramming factor to the cell, wherein the reprogramming factor comprises ETS2 or a combination of ETS2 and Mesp1.

A further embodiment of the present invention provides a cardiac progenitor cell which has been induced by reprogramming a somatic cell, wherein reprogramming comprises delivery of a reprogramming factor comprising the ETS2 gene to the somatic cell. The somatic cell may be a normal human dermal fibroblast (NHDF), and the reprogramming factor may be ETS2 or Mesp1, or a combination thereof.

Still a further embodiment of the present invention provides a method of reprogramming a somatic cell to produce a cardiac progenitor cell, wherein reprogramming comprises delivery of a reprogramming factor comprising the ETS2 gene to the somatic cell. The somatic cell may be an NHDF, and the reprogramming factor may be ETS2 or Mesp1, or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1 shows a schematic map of lentivirus with the insertion of ETS2 and ELK4 full length DNA coding sequences. Functional elements and abbreviations: constitutive Ef-1 α promoter, multiple cloning sites (MCS), independent ribosome entry site (IRES) from human polio virus, coding sequence for enhanced green fluorescence protein (eGFP), Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), activation domain (AD), DNA-binding ETS domain. These plasmids were generated by standard recombinant DNA cloning techniques and then used to make lentiviruses to infect normal human dermal fibroblasts (NHDFs);

FIG. 2 shows (top panel) NHDFs treated with empty lentivirus (pWPI-GFP), pWPI-ELK4-GFP lentivirus, or pWPI-ETS2-GFP lentivirus, and (bottom panel) expression levels of GAPDH, NANOG, OCT3/4, SOX2 in cells infected with empty virus, virus carrying ETS2, virus carrying ELK4, or uninfected NHDF passage 3 (NHDF-P3);

FIG. 3 shows immunofluorescence staining with antibody to a stem cell marker NANOG in NHDF-P3 cells infected

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with lentivirus carrying ETS2, but not ELK4 or empty vector (top panel). A protein blot using anti-ETS2 antibody revealed expression of ETS2 in NHDFs infected with lentivirus carrying ETS2 for 4 weeks, while NHDFs infected with empty lentivirus showed no expression (bottom left panel). Immunofluorescence staining with anti-ETS2 antibody confirmed the induced expression in the cells infected with ETS2 lentivirus (bottom right panel);

FIG. 4 shows induction of stem cell markers NANOG, OCT3/4, SOX2, REX1 and c-MYC in stem cell-like colonies after 4 weeks in culture. The fluorescent colors (colors not shown in the figures) are as follows: DAPI (blue), GFP (green), NANOG and OCT3/4 (red), a mixture of all three colors is observed in the “merge” panels. Color fluorescent images are available upon request;

FIG. 5 shows flow cytometry demonstrating the percent of 119 human embryonic stem cells (A), uninfected NHDFs (B), or ETS2-infected cells (C) that stained for the stem cell surface marker SSEA-3. Negative controls, black lines; SSEA-3 staining, gray lines;

FIG. 6 shows flow cytometry demonstrating the percent of H9 human embryonic stem cells (A), uninfected NHDFs (B), or ETS2-infected cells (C) that stained for the stem cell surface marker Tra-1-81. Negative controls, black lines; Tra-1-81 staining, gray lines;

FIG. 7 shows images of EPS cells after infection with Mesp1 lentivirus. Cells were stained with DAPI to visualize nuclei (left panels) and with specific antibodies to ISL1, NKX2.5, GATA4, MEF2C, TNT and MHC3 to visualize indicated cardiac progenitor proteins (right panels). Panels in the middle show phase contrast images of cells. The fluorescent colors are as follows: DAPI (blue), protein-specific staining (red). Color fluorescent images are available upon request;

FIG. 8 shows that expression of NKX2.5, ISL1, GATA4, MEF2C, TBX5, MHC3, TNT, MLC2, CX43 and CX45, detected using RT-PCR, was only induced by the combination of ETS2 and Mesp1. Neither ETS2 or Mesp1 alone are capable of inducing these cardiogenic genes in NHDFs;

FIG. 9 shows induction of sequential de novo cardiac progenitor program. NHDFs were infected with ETS2 lentivirus, grown for 4 weeks, infected with Mesp1 lentivirus and cultured for 7 days, then aggregated by the hang-drop procedure and plated on a gelatin-coated dish;

FIG. 10 shows activation of cardiac progenitor program gene expression, measured by fluorescence of the reporter protein Red-Tomato which is expressed only when the cardiac progenitor factor NKX2.5 is expressed. The fluorescent colors (colors not shown in the figures) are as follows: GFP (green), Red (red), a mixture of the two colors is observed in the “merge” panel;

FIG. 11 shows flow cytometry of cardiac progenitor cells obtained from NHDFs by infection with ETS2 and Mesp1 lentivectors and sorted for either GFP or GFP and reporter protein Red-Tomato;

FIG. 12 shows display of endothelial and cardiac cell surface markers CD31, CD34 and CD144 in cardiac progenitor cells after 9 days in culture; and

FIG. 13 shows the data on rhythmic beating in reprogrammed cardiac progenitor cells. EPS cells were infected with Mesp1 lentivirus, as well as with virus carrying a myosin heavy chain promoter driving the puromycin resistance gene. To select cardiac progenitor cells resistant to antibiotic, cells were treated with 50 ug/ml puromycin. After 9 days, rhythmic beating in the cell cultures was observed and captured by video microscopy and converted into MPEG videos. Beats per cultured aggregate per dish were counted for 20 sec and

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then multiplied by 3, resulting in beats per one minute. Three separate measurements were done per aggregate in a tissue culture dish or well.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

One embodiment of the present invention relates to the modulation of cell differentiation using heterologous gene expression. Some embodiments of the invention relate to a method for inducing a cardiac progenitor cell by delivering a reprogramming factor to the cell, wherein the reprogramming factor comprises ETS2 or a combination of ETS2 and Mesp1.

An embodiment of the present invention provides a method for inducing a cardiac progenitor cell by reprogramming a somatic cell, wherein reprogramming comprises delivery of a reprogramming factor comprising a single heterologous gene to the somatic cell. The somatic cell may be a fibroblast, preferably a normal human dermal fibroblast. The heterologous gene may be ETS2. The heterologous gene may comprise the human ETS2 coding sequence (SEQ ID NO:9) or the ETS2 gene (SEQ ID NO:7), or the heterologous gene may encode the human ETS2 protein sequence (SEQ ID NO:8). The induced stem-like cell may exhibit cardiogenesis or other characteristics of cardiac progenitor cells as a result of programming, including the expression of cardiac progenitor factors such as NKX2.5, ISL1, MEF2C, dHAND and GATA4, or rhythmic beating.

Another embodiment of the present invention provides a method for inducing a cardiac progenitor cell by reprogramming a somatic cell, wherein reprogramming comprises delivery of a reprogramming factor comprising two heterologous genes to the somatic cell. The somatic cell may be a fibroblast, preferably a normal human dermal fibroblast. The heterologous genes may be ETS2 and Mesp1. The heterologous genes may comprise the human ETS2 coding sequence (SEQ ID NO:9), the ETS2 gene (SEQ ID NO:7), or a DNA sequence encoding the human ETS2 protein sequence (SEQ ID NO:8) and the mouse Mesp1 coding sequence (SEQ ID NO:6), the mouse Mesp1 gene (SEQ ID NO:4), or a DNA sequence encoding the mouse Mesp1 protein sequence (SEQ ID NO:5). The induced stem-like cell may exhibit cardiogenesis or other characteristics of cardiac progenitor cells as a result of programming, including the expression of cardiac progenitor factors such as NKX2.5, ISL1, MEF2C, dHAND and GATA4, or rhythmic beating.

Yet another embodiment of the present invention, reprogramming of a somatic cell, may be accomplished by delivering a reprogramming factor to the somatic cell using a recombinant vector. The reprogramming factor may also be delivered using a lentiviral transduction system to express the reprogramming factor in the somatic cell. In these embodiments, the reprogramming factor may be ETS2 and Mesp1.

A further embodiment of the present invention provides a somatic cell which has been reprogrammed, wherein reprogramming comprises delivery of a reprogramming factor comprising a single heterologous gene or multiple heterologous genes to the somatic cell. The somatic cell may be a fibroblast, preferably a normal human dermal fibroblast. The heterologous genes may be ETS2 or the multiple heterologous genes may be ETS2 and Mesp1. The induced stem-like cell may exhibit cardiogenesis or other characteristics of cardiac progenitor cells as a result of programming, including the expression of cardiac progenitor factors such as NKX2.5, ISL1, MEF2C, dHAND and GATA4, or rhythmic beating.

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EXAMPLE 1

Selection of a Reprogramming Factor

It was noted that the ETS domain (FIG. 1), a highly conserved DNA-binding domain, is capable of binding to a 5'-GGA(A/T)-3' DNA core motif found on the promoters of many stem cell marker genes. The expression of ETS2 is linked to immortalization of cells, mediation of oncogenesis, and enhancement of telomerase activity.

ETS2 and ELK4, an ETS family gene homologous to ETS2 in its DNA-binding region, were transduced using lentiviral vectors into NHDF-P3. Within one week, fibroblasts transduced with lentiviral vectors containing ETS2 were replaced with highly proliferative small rounded cells. These highly proliferative cells were not observed in controls transduced with empty lentivirus, or in the fibroblasts transduced with lentiviral vectors containing ELK4 (FIG. 2-3).

EXAMPLE 2

Lentiviral Transduction System

FIG. 1 shows a schematic map of lentivirus with the insertion of ETS2 (SEQ ID NO:9) and ELK4 (SEQ ID NO:3) DNA coding sequences. Note that only ETS2 has a pointed domain. These plasmids were used to make lentiviruses to infect NHDFs. The ETS2 full-length sequence (SEQ ID NO:7) comprises an ETS2 coding sequence (SEQ ID NO:9) encoding an ETS2 protein sequence (SEQ ID NO:8). The ELK4 full-length sequence (SEQ ID NO:1) comprises an ELK4 coding sequence (SEQ ID NO:3) encoding a protein sequence (SEQ ID NO:2).

The empty lentivirus vector pWPI-eGFP was a gift from Dr. D. Trono (Ecole Polytechnique Fédérale de Lausanne, Switzerland). cDNA for cloning the human ETS2 and ELK4 genes (Clone IDs 3852274 and 4364006) were obtained from Open Biosystems, whereas the Mesp1 cDNA was a gift from Dr. Y. Saga (National Institute of Genetics, Mishima, Japan). The consensus Kozak sequence for initiation of protein translation and the epitope HA-tag were added respectively to the 5'- and 3'-ends of ETS2, ELK4 and Mesp1 coding sequences by PCR cloning.

Lentivirus packing and infection proceeded as follows: Seeded 293FT cells in 6-cm dishes were transfected with either pWPI-eGFP, or pWPI-ELK4-eGFP (human ELK4 coding sequence, SEQ ID NO:3), or pWPI-ETS2-eGFP (human ETS2 coding sequence, SEQ ID NO:9), or pWPI-Mesp1-eGFP (mouse Mesp1 sequence, SEQ ID NO:6), or SMPU-alphaMHC/puro-Rex1/Blast (gift from Dr. M. Mercola, Burnham Institute for Medical Research, La Jolla, Calif.). 4.5 ug of either construct was mixed in a solution of 458 ul of serum-free Dulbecco-modified Eagle medium (DMEM) and 27.5 ul of Fugene (Roche), 2.8 ug of packing

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vector psPAX2 and 1.9 ug of envelope vector pMD2.G for 25 min at room temperature. Afterwards the mix was added to 293FT cells grown in DMEM, phenol red-free (Invitrogen) supplemented with 10% FBS (heat-inactivated), 0.1 mM MEM non-essential amino acids, 1 mM sodium pyruvate and 6 mM L-glutamate. After 24-26 hrs in culture, medium with viral particles was collected for 3 days and used for infection.

Collected medium was used to infect NHDFs grown in Fibroblast Basal Medium (FBM, Lonza) until 80% confluency. Before transfection, cells were reseeded in 6-cm Petri dishes at a density of 2.5×10^6 cell/dish, the medium was changed to StemPro and the viral particles and polybrene (8 ug/ml final concentration) were added. To increase the efficiency of infection, the procedure was repeated within 48 hours. All cells were grown at 37° C. and 5% CO₂.

EXAMPLE 3

Gene Expression in Reprogrammed Cells

FIG. 2 shows that ETS2 lentivirus but not ELK4 lentivirus induced stem cell appearance and the induction of stem cell marker proteins, NANOG, OCT3/4 and SOX2 within 7 days of culture. The top panel of FIG. 2 shows NHDFs (Lonza, USA, cc-2509) grown under FBM, supplemented (supplements provided by Lonza) with hFGF-beta, insulin, gentamycin/amphotericin and 2% FBS, to a confluence of ca. 80% before viral infection. Empty pWPI-eGFP and pWPI-ELK4-eGFP and pWPI-ETS2-eGFP lentiviruses were used to separately infect NHDF-P3. Infected and non-infected NHDF-P3 cells were grown under human induced pluripotent medium StemPro hES SFM (Invitrogen) over collagen-coated Petri dishes for 7 days. The green fluorescent protein cloned into the lentiviral vectors was expressed in the infected cells, as revealed by the green fluorescence microscopy. During this culture period, morphological changes were observed in which ETS2-infected NHDFs changed their appearance from elongated pleomorphic fibroblastic shapes to rounded "stem-like cells". In comparison, NHDFs infected with an empty or ELK4 lentiviral vectors did not alter cell shape.

The bottom panel of FIG. 2 shows the reverse transcription PCR (RT-PCR) analysis of reprogrammed cells. Cells were washed in chilled PBS and RNA was isolated with Qiagen RNeasy Kit. RNA was transcribed using MMLV reverse transcriptase (Invitrogen), and PCR amplification (30 cycles) was performed for GAPDH, NANOG, OCT3/4, SOX2 (refer to Table 1 for primer sets) using LA16 polymerase mix. This enzyme mix was prepared using 15 ul of KlenTaq1 (25 units/ul, Ab Peptides, St Louis, Mo.) and 1 ul Pfu (2.5 units/ul, Stratagene, La Jolla, Calif.). Amplified DNA samples were then electrophoresed on 2% agarose gel and ethidium bromide staining revealed the induced expression of stem cell marker genes NANOG, OCT3/4 and SOX2 in ETS2-infected cells but not in NHDF-P3 or cells infected with either ELK4 or empty lentiviruses.

TABLE 1

Primers for Cardiac Progenitor Study			
Primer	SEQ ID NO	Seq	Product-size
GapdhFP ctrl exp	11	TGTTGCCATCAATGACCCCTT	202
GapdhRP ctrl exp	12	CTCCACGACGTACTCAGCG	
hNanogFP ctrl exp	13	CAGAAGGCCTCAGCACCTAC	225
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TABLE 1-continued

Primers for Cardiac Progenitor Study			
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Sox2 HPB R	18	CGAGTAGGACATGCTGTAGGT	
hRex1FP ctrl exp	19	GCTGACCACCAGCACACTAGGC	298
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c-Myc HPB R	22	TGGACGGACAGGATGTATGC	
hKlf4FP ctrl exp	23	ATGGCTGTCAGCGACGCGTGCTC	293
hKlf4RP ctrl exp	24	CGTTGAACTCCTCGGTCTCTCTCC	
Nkx 2.5 FP	25	ccctgaccgatcccacctcaac	358
Nkx 2.5 RP	26	GGCGGGCGACGCGGAGATAGC	
Mesp 1 FP	27	tcgaagtgggttccttggcagac	162
Mesp 1 RP	28	CCTCTGCTTGCTCAAAGTGTC	
Mesp 2 FP	29	CGCTGCGCTGGCCATCCGCTACAT	113
Mesp 2 RP	30	GCCCAAGGGGACCCCGGAC	
Mef2c FP	31	gcaccagtgcagggaaacggg	202
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Sox 17 FP	33	gcggcgcaagcaggtgaag	205
Sox 17 RP	34	ACTCTGGCAGTCGCGGTAGTGGC	
FoxA2 FP	35	ctgaagccggaacaccactacgc	214
FoxA2 RP	36	TCCAGGCCGTTTTGTTCTGTGAC	
FGF8 FP	37	agctcagccgcccctcatccg	313
FGF8 RP	38	AGCCCTCGTACTTGGCATTCTGC	
MyoD FP	39	AggggctaggttcagctttctcG	240
MyoD RP	40	CTCCTGCTCTGGCAAAGCAACTC	
BMP2 HBP FP	41	ACCTTTATGGAGGGAACCCA	201
BMP2 HBP RP	42	CCGGATCTGGTTC AAGCATGA	
hTert HBP FP	43	AACCTTCTCAGCTATGCC	210
hTert HBP RP	44	GCGTGAAACCTGTACGCCT	
Islet-1 HPB F	45	GTGGAGAGGGCCAGTCTAGG	250
Islet-1 HPB R	46	CCGTCACTCTACCAGTTGCT	
Troponin T HPB F	47	GAGTTGCAGGCGCTGATTG	229
Troponin T HPB R	48	TCTGGATGTAACCCCAAAATG	
Dhand HPB F	49	ATGAGTCTGGTAGGTGGTTTTCC	205
Dhand HPB R	50	CATACTCGGGCTGTAGGACA	
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T HPB R	52	TCTCTGGTGTGTTCC TAGACG	
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T5 HPB R	54	TGGCAGCCATGAGAGTAGA	
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ETS-2 HPB R	56	AATGTCACCCACAAAGTCAGG	
Dkk-1 FP	57	ATTCCAACGCTATCAAGAACC	384
Dkk-1 RP	58	CCAAGGTGCTATGATCATTACC	
TBX 20 FP	59	tccagattctccttttacgg	190
TBX 20 RP	60	ttcagaacttcaggttgagca	
SM actin HBP F	61	CGGTGCTGTCTCTATGCC	156
SM actin HBP R	62	CACGCTCAGTCAGGATCTTCA	
hGATA1 F	63	AGAAGCGCCTGATTGTGAGTA	229
hGATA1 R	64	AGAGACTTGGTTGTCCAGAA	

TABLE 1-continued

Primers for Cardiac Progenitor Study			
Primer	SEQ ID NO	Seq	Product-size
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hGATA2 R	66	CATCTTCATGCTCTCCGTCAG	
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TBX3-2 F	69	TAAAGTGAGATGTTCTGGGCTG	298
TEX3-2 R	70	ACTATAATTCCCTGCCACGTA	
TBX4 F	71	TGACCATCGCTACAAGTTCTGT	163
TEX4 R	72	GGTGGTTGTTTGTCTCAGCTTCAG	
TBX6 F	73	ACACCCCTAAACTGGATTGCT	229
TEX6 R	74	CCTCCAGCTTGGTGATGAT	
TBX10 F	75	CCTCGGCATACTGCACCC	208
TEX10 R	76	ATTCTCCACAGAGGCTTCA	
TBX18 F	77	GCCCCTGCTGACTATTCTGC	227
TEX18 R	78	CTGCATGGATAAGCTGGTCTG	
TBX19 F	79	AAGAATGGCAGACGGATGTTT	204
TEX19 R	80	CCGGTGAATGTAGACGCAG	
RUNX2 F	81	CGGCAAAATGAGCGACGTG	268
RunX2 R	82	CACCGAGCACAGGAAGTTG	
LMO2 F	83	GGCCATCGAAAGGAAGAGCC	221
LMO2 R	84	GGCCAGTTTGTAGTAGAGGC	
TAU F	85	CCCCTGGAGTTCACGTTTCAC	240
TAU R	86	GCGAGCTTTGAGTTGAGGGA	

EXAMPLE 4

35

Stem Cell Marker Proteins in Reprogrammed Cells

FIG. 3 shows immunofluorescence staining of infected NHDF-P3 with antibody to the stem cell marker NANOG. Induced NANOG staining is revealed in ETS2-infected cells but not in ELK4- or empty vector-infected cells. Also, a protein blot using ETS2-specific antibody revealed its expression in cells infected with ETS2 lentivirus but not in NHDFs infected with empty lentivirus.

FIG. 4 shows ETS2 lentivirus-induced stem cell-like colonies and the induction of stem cell marker proteins NANOG, OCT3/4, SOX2, REX1 and c-MYC after 4 weeks in culture. The top left panel shows that NHDFs infected with ETS2 virus converted to colonies of small rounded cells highly reminiscent of cultured murine and human embryonic stem cells. Note that colonies were GFP-labeled through the infection with ETS2 lentivirus. Non-infected fibroblasts failed to round and did not stain for GFP or form cellular colonies.

The right top panel of FIG. 4 shows the expression of stem cell marker genes NANOG, OCT3/4, SOX2, REX1, KLF4 and c-MYC in ETS2-induced cellular colonies analyzed by RT-PCR. Underneath is graphic representation of quantitative RT-PCR for NANOG, OCT3/4, SOX2 and c-MYC in ETS2-infected cells.

The lower panels of FIG. 4 confirm the presence of NANOG and OCT3/4 proteins in ETS2-induced cellular colonies by immunofluorescence staining with specific antibodies.

EXAMPLE 5

Characteristics of Cells Transduced with ETS2

FIG. 5 shows that approximately 97% of H9 human embryonic stem cells stained for the stem cell surface marker SSEA-3 (Panel A). Over 60% of ETS2-infected cells also displayed surface marker SSEA-3 (Panel C), in comparison to less than 2% staining of the NHDFs infected with empty lentivirus (Panel B). Thus, ETS2 efficiently converted NHDFs into cells with the SSEA-3 surface marker resembling human embryonic stem cells.

Flow cytometry was done using a BD Biosciences LSR II analyzer. Confluent colony-forming cells were dissociated by trypsin, washed with PBS and diluted to a concentration of 5×10^6 cells/ml PBS in 8 samples (100 ul each). Thereafter 10 ul of normal human serum was used for blocking for 5 min. Antibodies to SSEA-3-PE (Becton Dickinson) were diluted and added according to manufacturer's specifications, and incubated for 1 hr at 4° C. Then 400 ul PBS was added and the mixture was spun down and half of the supernatant was removed and 200 ul PBS was added and assayed by flow cytometry.

FIG. 6 shows flow cytometry of cells stained for the stem cell surface marker Tra-1-81 performed as for SSEA-3. Approximately 45% of H9 human embryonic stem cells stained for Tra-1-81 (Panel A). No Tra-1-81 staining (Panel B) was detected for NHDFs infected with empty lentivirus (not carrying a heterologous gene). Infection with ETS2 virus gave rise to approximately 39% cells displaying embryonic stem cell marker Tra-1-81 (Panel C). This indicates that ETS2 efficiently converted NHDFs into cells with the Tra-1-81 surface marker resembling human embryonic stem cells.

OCT3/4, NANOG and SOX2 gene transcripts were observed only after NHDF-P3 were transduced with lentiviral vector containing ETS2 but not after transduction with empty lentivector or vector containing ELK4. OCT3/4, NANOG and SOX2 transcripts were visualized (FIG. 2) and NANOG induction was visualized using immunofluorescence (FIG. 3).

Lentiviral transduction of NHDF-P3 cells with ETS2 resulted in whole populations which showed robust ETS2 expression over 4 weeks visualized by protein blots (FIG. 3). These ETS2-transduced cells formed large green fluorescent colonies similar to those of pluripotent ES and/or induced pluripotent stem (iPS) cells.

Reprogramming of fibroblasts with ETS2 resulted in strong expression of the pluripotent marker genes NANOG, OCT3/4, SOX2 and c-MYC measured by both RT-PCR and quantitative PCR and immunostaining. Additionally, flow cytometry shows that ETS2 efficiently converted NHDFs into cells with surface markers SSEA-3 and Tra-1-81 resembling human embryonic stem cells. Thus, these ETS2-treated human fibroblast cells resemble iPS cells in their ability to express pluripotent stem cell marker proteins. These cells were therefore named "EPS" cells.

EXAMPLE 6

Combination of ETS2 and Mesp1 Induces De Novo Cardiac Progenitor Program in Fibroblasts

Next, EPS cells were subjected to lentiviral transduction with mouse Mesp1. The resulting EPS cells expressing Mesp1 could be induced to form embryoid bodies using protocols for forming embryoid bodies from ES cells. Plated cellular aggregates were further treated with activin and BMP4 for 4 days and then examined at 10 days. Constitutive expression of stem cell markers continued even after the transduction with Mesp1 and addition of growth factor morphogens.

Robust induction of the cardiac progenitor factors ISL1, NKX2.5, GATA4, MEF2C, TNF and MHC was observed by immunostaining only in the EPS cells infected with lentivirus expressing Mesp1. FIG. 7 shows cells stained with DAPI to visualize nuclei (left panels), phase contrast images (middle) and cells stained with specific antibodies to visualize indicated proteins (right panels).

FIG. 8 shows that the Mesp1 infection of EPS cells induces de novo cardiac progenitor program in cell that were originally NHDFs. Cardiac progenitor cells post-aggregation were plated for a week and then taken for RNA isolation. RNA was transcribed using MMLV reverse transcriptase, and PCR amplification for 30 cycles was performed for GAPDH, NKX2.5, ISL1, GATA4, MEF2C, TBX5, MHC3, TNF, MLC2, CX43 and CX45 (refer to Table 1 for primer sets), using LA-16 polymerase mix.

FIG. 8 shows that in EPS cells grown for 4 weeks, aggregated and plated for 7 days, no transcripts were observed for markers of early heart development by RT-PCR. Similarly, no appreciable expression of the early heart development markers was detected after infection of NHDFs with Mesp1 alone. However, Mesp1 addition to EPS cells induced robust expression of cardiac mesoderm progenitor markers including NKX2.5, ISL1, GATA4, MEF2C, TBX5, and cardiac contractile protein gene expression including alpha MHC and troponin T.

FIG. 9 shows that the Mesp1 infection of EPS cells induced sequential de novo cardiac progenitor program as determined by RT-PCR. NHDF-P3 cells were infected with ETS2 lentivi-

rus, grown for 4 weeks, then infected with Mesp1 lentivirus, aggregated and plated for 7 days. RNA was isolated daily and analyzed by RT-PCR for expression of cardiogenic genes.

FIG. 10 shows that the Mesp1 infection of EPS cells induced de novo cardiac program gene expression as shown by the appearance of Red Tomato fluorescence staining from the reporter construct NKX2.5-Red Tomato. NKX2.5/Smad/GATA enhancer, which is activated in cardiac progenitors, was linked to the minimal HSP68 promoter adjacent to the puromycin resistance gene and an IRES sequence and the powerful reporter td-Tomato (cDNA was a gift from Dr. R. Tsien, University of California, San Diego). As shown above, GFP staining resulted from ETS2 and Mesp1 lentivirus infection of NHDFs. The Red Tomato fluorescence is consistent with the ETS2/Mesp1 driven conversion to cardiac progenitors by the induction of NKX2.5 gene expression. Note the appearance of many triangular and rectangular appearing cells that are highly similar in shape to cardiac myocytes.

FIG. 11, Panel A shows a summary of FACS sorting of cardiac progenitor cells obtained from sequential treatment of NHDFs with ETS2 lentivirus and then of the resultant EPS cells with Mesp1 lentivirus. Flow cytometry was done using a BD Biosciences LSR II analyzer. Confluent colony-forming cells were dissociated by trypsin, washed with PBS and diluted to a concentration of 5×10^6 cells/ml PBS in 8 samples. Panel A shows GFP stained cells accounting for the total lentivirus infection, since each virus is GFP-tagged. Panel B shows that approximately 19% of the ETS2 and Mesp1 infected cells were both stained by GFP and Red-Tomato (shaded area). As evidenced by activation of a key cardiogenic reporter NKX2.5 Red-Tomato, ETS2 and Mesp1 efficiently convert NHDFs into cells with characteristics resembling cardiac progenitors.

FIG. 12 shows that approximately 10 to 40% of ETS2 and Mesp1 infected NHDF cells display endothelial and cardiac cell surface markers, CD31, CD34 and CD144 after 9 days in culture. This is an additional line of evidence that ETS2 and Mesp1 efficiently converted NHDFs into cells with characteristics resembling embryonic endothelial and cardiac myocytes.

EXAMPLE 7

Cardiac Properties of Reprogrammed Cells

Lentiviral transduction of a puromycin selectable system using a lentiviral cardiac-specific alpha-myosin heavy chain (alpha-MHC) promoter and enhancer linked to the puromycin resistance gene resulted in enrichment of the cardiac progenitor cells and subsequent observation of a rhythmic beating of the transduced cells, similar to that observed in cardiac myocytes.

A myosin heavy chain promoter driving the puromycin selectable gene construct was transduced into NHDFs which were then sequentially transduced with ETS2 and Mesp1. Cellular aggregates obtained during hang-drop embryoid body formation were then treated with 50 ug/ml puromycin to select cells resistant to puromycin and therefore having the active cardiac specific alpha-MHC promoter.

After 9 days beating in the cell cultures was observed and captured using video microscopy and converted into MPEG videos. Beating per cultured aggregate per dish was counted for 20 sec and then multiplied by 3 for beats per one minute (FIG. 13). Three separate measurements were done per aggregate in a tissue culture dish or well.

Reprogramming of EPS cells with Mesp1 resulted in strong expression of cardiac progenitor genes as determined

by RT-PCR and immunostaining. Additionally, flow cytometry showed that Mesp1 efficiently converted EPS cells into cells with surface markers CD31, CD34 and CD144 resembling human cardiac cells. Finally, rhythmic beating was observed in the cell cultures. This completed the conversion from skin fibroblasts to terminally differentiated cardiogenic cells.

REFERENCES CITED

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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ccctcatcca gactgtacca ttccaacct ccttggaagg aggcgcccga tagggtacac 1020
gctctaaaga tgaagcagcc acaagctttg cctggtgtgt atttatttat ttgtgaataa 1080
accgattgtg ctagtgtcaa aacctggata gtcgatccac ta 1122

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<210> SEQ ID NO 5
<211> LENGTH: 243
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mouse MesP1 protein sequence

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<400> SEQUENCE: 5

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Met Ala Gln Pro Leu Cys Glu Pro Arg Ser Glu Ser Trp Ile Leu Ser
1           5           10           15
Pro Ala Gly Arg Gln Pro Pro Met Pro Ser Asp Gly Asn Ser Val Cys
20           25           30

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Ser Pro Ala Trp Ser Ser Asp Pro Trp Asp Gly Ala Gln Ala Ser Ser
 35 40 45
 Pro Ala Pro Pro Cys Ala Arg Pro Ala Arg Arg Ala Gly Thr Pro Gly
 50 55 60
 Arg Arg Gly Thr His Gly Ser Arg Leu Gly Ser Gly Gln Arg Gln Ser
 65 70 75 80
 Ala Ser Glu Arg Glu Lys Leu Arg Met Arg Thr Leu Ala Arg Ala Leu
 85 90 95
 His Glu Leu Arg Arg Phe Leu Pro Pro Ser Val Ala Pro Thr Gly Gln
 100 105 110
 Asn Leu Thr Lys Ile Glu Thr Leu Arg Leu Ala Ile Arg Tyr Ile Gly
 115 120 125
 His Leu Ser Ala Val Leu Gly Leu Ser Glu Asp Asn Leu Arg Arg Gln
 130 135 140
 Arg His Ala Val Ser Pro Arg Gly Cys Pro Leu Cys Pro Asp Ser Asp
 145 150 155 160
 Leu Ala Gln Ser Gln Ser Leu Gly Pro Gly Leu Ser Pro Ala Val Cys
 165 170 175
 Ser Gly Val Ser Trp Gly Ser Pro Pro Ala Tyr Pro Arg Pro Arg Val
 180 185 190
 Ala Ala Glu Ser Trp Asp Pro Ser Phe Gln Tyr Ala Glu Thr Ala Ser
 195 200 205
 Gln Glu Arg Gln Glu Met Glu Pro Ser Pro Ser Ser Pro Leu Phe Ser
 210 215 220
 Ser Asp Met Leu Ala Leu Leu Glu Thr Trp Thr Pro Pro Gln Glu Trp
 225 230 235 240
 Pro Pro Ala

<210> SEQ ID NO 6
 <211> LENGTH: 732
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: mouse MesP1 coding sequence

<400> SEQUENCE: 6

atggcccagc ccctgtgcga gccgcgctcc gagtctctgga tcctgagtcc cgctgggtcgg 60
 cagccaccga tgccttccga tgggaacagc gtctgtctccc cagcctgggtc ctcggaaccg 120
 tgggacgggtg cccaggccag cagccctgca ccacctgcg cccgcccggc cgggcgtgct 180
 gggacccccg gtaggcgcgg gacgcacggt agccgcctgg gtagcggaca gcggcagagc 240
 gccagcgagc gggagaagct acgtatgcmc aactcgcgcc gcgcgctgca cgagctgcmc 300
 cgcttcttgc cgccatccgt ggcaccaacc ggcagaacc tgaccaagat cgagacgctg 360
 cgctgggcca tccgctacat tggccaactg tcggctgtgc tgggactcag cgaggacaac 420
 ctccggcgac agcggcacgc ggtgtcacct cgaggctgcc cgctgtgccc cgacagcgac 480
 ctggcgcagt cgcagtcact cggtcctggg ttaagccccg ccgtctgcag cggggtgtcg 540
 tggggatccc cgctgccta ccctagacc cgagtcgccc cagaatcgtg ggacctatcg 600
 ttccagtagc cagaacacgc atcccaggaa aggcaggaaa tggagcccag tcctcatct 660
 ccgctcttca gcagcgacat gctggctctt ctagaacct ggacgcccgc gcaggagtgg 720
 ccgctgctct ga 732

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<210> SEQ ID NO 7
<211> LENGTH: 2500
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human ETS2 gene sequence

<400> SEQUENCE: 7
gtgtcgcctcc agctcagagc tcccggagcc gcccggccag cgtccggcct ccctgatcgt    60
ctctggcccg cgccctcgcc ctcgcccggc gcgcaccgag cagccgcggg cgccgagcag    120
ccaccgtccc gaccaagcgc cggccctgcc cgcagcggca ggatgaatga tttcggaatc    180
aagaatatgg accaggtagc ccctgtggct aacagttaca gagggacact caagcgcag    240
ccagcctttg acacctttga tgggtccctg tttgctgttt ttccttctct aaatgaagag    300
caaacactgc aagaagtgcc aacaggcttg gattccattt ctcatgactc cgccaactgt    360
gaattgcctt tgttaacccc gtgcagcaag gctgtgatga gtcaagcctt aaaagctacc    420
ttcagtggct tcaaaaagga acagcggcgc ctgggcattc caaagaacct ctggctgtgg    480
agtgagcaac aggtatgcca gtgcttctc tgggcccaca atgagttcag tctgggtgaa    540
gtgaatctgc agaggttcgg catgaatggc cagatgctgt gtaaccttgg caaggaacgc    600
tttctggagc tggcacctga ctttgtgggt gacattctct gggaacatct ggagcaaatg    660
atcaaagaaa accaagaaaa gacagaagat caatatgaag aaaattcaca cctcacctcc    720
gttctcattt ggattaacag caatacatta ggttttgcca cagagcaggc gccctatgga    780
atgcagacac agaattaccc caaaggcggc ctccctggaca gcatgtgtcc ggccctccaca    840
cccagcgtac tcagctctga gcaggagttt cagatgttcc ccaagtctcg gctcagctcc    900
gtcagcgtca cctactgctc tgcagtcag gacttcccag gcagcaactt gaatttgctc    960
accaacaatt ctgggacgcc caaagaccac gactcccctg agaacggtgc ggacagcttc    1020
gagagctcag actccctcct ccagtcctgg aacagccagt cgtccttctg ggatgtgcaa    1080
cgggttcctt ccttcgagag cttcgaagat gactgcagcc agtctctctg cctcaataag    1140
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cagtttctcc tggagctgct atcagacaaa tcctgccagt cattcatcag ctggactgga    1320
gacggatggg agtttaagct cgcgcacccc gatgaggtgg cccgcgggtg gggaaagag    1380
aaaaataagc ccaagatgaa ctacgagaag ctgagccggg gcttacgcta ctattacgac    1440
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cagaacttgc tggggttcac gcccgaggaa ctgcacgcca tcctgggctg ccagcccgcac    1560
acggaggact gaggtcgccg ggaccacctt gagccggccc caggctcgtg gactgagtg    1620
gaagcccatc ctgaccagct gctccgagga cccaggaaaag gcaggattga aaatgtccag    1680
gaaagtggcc aagaagcagt ggccttattg catcccaaac cagcctctt gaccaggctg    1740
cctcccttgt ggcagcaacg gcacagctaa ttctactcac agtgctttta agtgaaaatg    1800
gtcgagaaaag aggcaccagg aagccgtcct ggcgcctggc agtccgtggg acgggatgg    1860
tctggctggt tgagattctc aaaggagcga gcatgtcgtg gacacacaca gactattttt    1920
agattttctt ttgccttttg caaccaggaa cagcaaatgc aaaaactctt tgagagggt    1980
ggagggtggg aaggaaacaa ccatgtcatt tcagaagtta gtttgatat attattataa    2040
tcttataaatt gttctcagaa tcccttaaca gttgtattta acagaaattg tatattgtaa    2100

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ttaaataa ttatataact gtatttgaaa taagaattca gacatctgag gttttatttc 2160
atthttcaat agcacatatg gaattttgca aagatttaat ctgccaaggg cggactaaga 2220
gaagttgtaa agtatgtatt atttacattt aatagactta cagggataag gcctgtgggg 2280
ggtaatccct gctttttgtg tttttttggt tgtttgtttg tttgtttttg gggggttttc 2340
ttgccttggg tgtctggcaa ggactttgta catttggggag tttttatgag aaacttaaat 2400
gttattatct gggettatat ctggcctctg cttttctcct taattgtaaa gtaaaagcta 2460
taaagcagta tttttcttga caaaaaaaaa aaaaaaaaaa 2500

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<210> SEQ ID NO 8

<211> LENGTH: 469

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: human ETS2 protein sequence

<400> SEQUENCE: 8

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Met Asn Asp Phe Gly Ile Lys Asn Met Asp Gln Val Ala Pro Val Ala
1           5           10          15
Asn Ser Tyr Arg Gly Thr Leu Lys Arg Gln Pro Ala Phe Asp Thr Phe
20          25          30
Asp Gly Ser Leu Phe Ala Val Phe Pro Ser Leu Asn Glu Glu Gln Thr
35          40          45
Leu Gln Glu Val Pro Thr Gly Leu Asp Ser Ile Ser His Asp Ser Ala
50          55          60
Asn Cys Glu Leu Pro Leu Leu Thr Pro Cys Ser Lys Ala Val Met Ser
65          70          75          80
Gln Ala Leu Lys Ala Thr Phe Ser Gly Phe Lys Lys Glu Gln Arg Arg
85          90          95
Leu Gly Ile Pro Lys Asn Pro Trp Leu Trp Ser Glu Gln Gln Val Cys
100         105         110
Gln Trp Leu Leu Trp Ala Thr Asn Glu Phe Ser Leu Val Asn Val Asn
115         120         125
Leu Gln Arg Phe Gly Met Asn Gly Gln Met Leu Cys Asn Leu Gly Lys
130         135         140
Glu Arg Phe Leu Glu Leu Ala Pro Asp Phe Val Gly Asp Ile Leu Trp
145         150         155         160
Glu His Leu Glu Gln Met Ile Lys Glu Asn Gln Glu Lys Thr Glu Asp
165         170         175
Gln Tyr Glu Glu Asn Ser His Leu Thr Ser Val Pro His Trp Ile Asn
180         185         190
Ser Asn Thr Leu Gly Phe Gly Thr Glu Gln Ala Pro Tyr Gly Met Gln
195         200         205
Thr Gln Asn Tyr Pro Lys Gly Gly Leu Leu Asp Ser Met Cys Pro Ala
210         215         220
Ser Thr Pro Ser Val Leu Ser Ser Glu Gln Glu Phe Gln Met Phe Pro
225         230         235         240
Lys Ser Arg Leu Ser Ser Val Ser Val Thr Tyr Cys Ser Val Ser Gln
245         250         255
Asp Phe Pro Gly Ser Asn Leu Asn Leu Leu Thr Asn Asn Ser Gly Thr
260         265         270
Pro Lys Asp His Asp Ser Pro Glu Asn Gly Ala Asp Ser Phe Glu Ser
275         280         285
Ser Asp Ser Leu Leu Gln Ser Trp Asn Ser Gln Ser Ser Leu Leu Asp

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290			295			300									
Val	Gln	Arg	Val	Pro	Ser	Phe	Glu	Ser	Phe	Glu	Asp	Asp	Cys	Ser	Gln
305					310						315				320
Ser	Leu	Cys	Leu	Asn	Lys	Pro	Thr	Met	Ser	Phe	Lys	Asp	Tyr	Ile	Gln
				325							330				335
Glu	Arg	Ser	Asp	Pro	Val	Glu	Gln	Gly	Lys	Pro	Val	Ile	Pro	Ala	Ala
				340							345				350
Val	Leu	Ala	Gly	Phe	Thr	Gly	Ser	Gly	Pro	Ile	Gln	Leu	Trp	Gln	Phe
		355						360							365
Leu	Leu	Glu	Leu	Leu	Ser	Asp	Lys	Ser	Cys	Gln	Ser	Phe	Ile	Ser	Trp
		370						375							380
Thr	Gly	Asp	Gly	Trp	Glu	Phe	Lys	Leu	Ala	Asp	Pro	Asp	Glu	Val	Ala
		385						390							400
Arg	Arg	Trp	Gly	Lys	Arg	Lys	Asn	Lys	Pro	Lys	Met	Asn	Tyr	Glu	Lys
				405							410				415
Leu	Ser	Arg	Gly	Leu	Arg	Tyr	Tyr	Tyr	Asp	Lys	Asn	Ile	Ile	His	Lys
				420							425				430
Thr	Ser	Gly	Lys	Arg	Tyr	Val	Tyr	Arg	Phe	Val	Cys	Asp	Leu	Gln	Asn
		435						440							445
Leu	Leu	Gly	Phe	Thr	Pro	Glu	Glu	Leu	His	Ala	Ile	Leu	Gly	Val	Gln
		450						455							460
Pro	Asp	Thr	Glu	Asp											
		465													

<210> SEQ ID NO 9

<211> LENGTH: 1410

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: human ETS2 coding sequence

<400> SEQUENCE: 9

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atgaatgatt tcggaatcaa gaatatggac caggtagccc ctgtggctaa cagttacaga    60
gggacactca agcgccagcc agcctttgac acctttgatg ggccctgtt tgctgttttt    120
ccttctctaa atgaagagca aacctgcaa gaagtgccaa caggcttggg ttccatttct    180
catgactccg ccaactgtga attgcctttg ttaaccccg tgcagcaaggc tgtgatgagt    240
caagccttaa aagctacctt cagtggcttc aaaaaggaac agcggcgccct gggcattcca    300
aagaaccctt ggctgtggag tgagcaacag gtatgccagt ggcttctctg gggcaccaat    360
gagttcagtc tggatgaact gaactctgag aggttcggca tgaatggcca gatgctgtgt    420
aaccttgcca aggaacgctt tctggagctg gcacctgact ttgtgggtga cattctctgg    480
gaacatctgg agcaaatgat caaagaaaac caagaaaaga cagaagatca atatgaagaa    540
aattcacacc tcacctccgt tcctcattgg attaacagca atacattagg ttttggcaca    600
gagcagggcg cctatggaat gcagacacag aattacccca aaggcggcct cctggacagc    660
atgtgtccgg cctccacacc cagcgtactc agctctgagc aggagtttca gatgttcccc    720
aagtctcggc tcagctccgt cagcgtcacc tactgctctg tcagtcagga cttcccaggc    780
agcaacttga atttgctcac caacaattct gggacgcccc aagaccacga ctcccctgag    840
aacggtgcgg acagcttoga gagctcagac tccctcctcc agtcctggaa cagccagtcg    900
tccttgctgg atgtgcaacg ggttccttcc ttcgagagct tcgaagatga ctgcagccag    960
tctctctgcc tcaataagcc aaccatgtct ttcaaggatt acatccaaga gaggagtgc    1020

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ccggtggagc aaggcaaac agttatacct gcagctgtgc tggccggctt cacaggaagt 1080
ggacctattc agctgtggca gtttctcctg gagctgctat cagacaaatc ctgccagtca 1140
ttcatcagct ggactggaga cggatgggag ttttaagctcg ccgacccoga tgagggtggc 1200
cgccggtggg gaaagaggaa aaataagccc aagatgaact acgagaagct gagccggggc 1260
ttacgctact attacgacaa gaacatcadc cacaagacgt cggggaagcg ctacgtgtac 1320
cgcttcgtgt gcgacctoca gaacttctgt gggttcacgc ccgaggaact gcacgccatc 1380
ctgggcgtcc agcccgacac ggaggactga 1410

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<210> SEQ ID NO 10
<211> LENGTH: 5143
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: human ETS sequence

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<400> SEQUENCE: 10

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agttgaaaag agaccacaga ctttgaggga agctcactca ggatctgctc tccggcaaag 60
tagtaagtga ggtgctgaga gcagaatgag ctactttgtg gattctgctg ggagcagccc 120
cgtcccttac tcagcgctc gtctctgagc ggtgaggcaa ggacctagca acacttatga 180
agatcctcga atgaactgtg gtttccagtc caattatcac cagcaaagac cttgctaccc 240
cttttgggat gagatggcaa ctcaggaagt tctactgggt cttgaacact gtgtctcaga 300
tatggaatgt gcagatgtcc cactattaac tccaagcagc aaagaaatga tgtctcaagc 360
atataaagct actttcagtg gtttactaa agaacagcaa cgactgggga tcccaaaaga 420
cccccgagcaggacagaaa cccatgttcg ggactgggtg atgtgggctg tgaatgaatt 480
cagcctgaaa ggtgtagact tccagaagtt ctgtatgaat ggagcagccc tctgcgccct 540
gggtaaagac tgctttctcg agctggcccc agactttgtt ggggacatct tatgggaaca 600
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ctatccagaa tcccgctata cctcggatta cttcattagc tatggattg agcatgccc 720
gtgtgttcca ccacggagc tctcagagcc cagcttcac acagagctct atcagacgct 780
ccatccatc agctcggaag agctcctctc cctcaagtat gagaatgact acccctcggc 840
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gctgcagccc atgctggagc tcaagccaga tgccgacgag tgatggcact gaaggggctg 1560
gggaaacct gctgagacct tccaaggaca gccgtgttg ttggactctg aattttgaat 1620
tgttattcta ttttttatt tccagaactc attttttacc ttcaggggtg ggagctaagt 1680

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cagttgcagc	tgtaatcaat	tgtgcgcagt	tgggaaagga	aagccaggac	ttgtggggtg	1740
ggtgggacca	gaaattcttg	agcaaatttt	caggagaggg	agaagggcct	tctcagaagc	1800
ttgaaggctc	tggcttaaca	gagaaagaga	ctaattgtgc	caatcatttt	taaaaatcat	1860
ccatgaaaa	gtgtcttgag	ttgtggaccc	attagcaagt	gacattgtca	catcagaact	1920
catgaaactg	atgtaaggca	attaatttgc	ttctgttttt	aggtctggga	gggcaaaaa	1980
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taatgggtaa	gagtagaacc	agggccgggt	atcaattatt	gcttgcctgt	tgcaaccag	3960
cataaaatca	ctttctcaaa	tcattccaccg	ttcctattaa	atztatgccg	gaaactctcc	4020

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gatttgtaga tagtgtatta gttaagacag ctttgctgat ctggccagat gttttttctc 4200
ctttgtccaa aggccagaga ccatcccagg aagagtggtg ggtggtttat aactggaaa 4260
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tcagttattg aacttttttt ttaagaata cttttttttt aagctgagaa gtatagggat 4800
gaaaagatg ttatatgtg tttgactatt ttccaactg tattttcata taatttatat 4860
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Mesp 2 FP primer

<400> SEQUENCE: 29
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<210> SEQ ID NO 30
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<220> FEATURE:
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<400> SEQUENCE: 30
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<210> SEQ ID NO 31
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<400> SEQUENCE: 31
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<210> SEQ ID NO 32
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<400> SEQUENCE: 32
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<211> LENGTH: 19
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<220> FEATURE:
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<210> SEQ ID NO 35
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 <220> FEATURE:
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 <400> SEQUENCE: 35

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 <400> SEQUENCE: 37

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<210> SEQ ID NO 38
 <211> LENGTH: 23
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<400> SEQUENCE: 44

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<400> SEQUENCE: 45

gtggagaggg ccagtctagg 20

<210> SEQ ID NO 46
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<400> SEQUENCE: 46

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 <211> LENGTH: 19
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<213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: Troponin T HPB F primer

 <400> SEQUENCE: 47

 gagttgcagg cgctgattg 19

 <210> SEQ ID NO 48
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 <212> TYPE: DNA
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 <220> FEATURE:
 <223> OTHER INFORMATION: Troponin T HPB R primer

 <400> SEQUENCE: 48

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 <210> SEQ ID NO 49
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 <220> FEATURE:
 <223> OTHER INFORMATION: Dhand HPB F primer

 <400> SEQUENCE: 49

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 <223> OTHER INFORMATION: Dhand HPB R primer

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 <400> SEQUENCE: 51

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 <210> SEQ ID NO 52
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 <400> SEQUENCE: 53

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 aaagctacct tcagtggtt c 21

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 ccaagtgct atgatcatta cc 22

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 <400> SEQUENCE: 59

 tccagattct ccttttaccg 20

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<223> OTHER INFORMATION: TBX 20 RP primer

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cggtgctgtc tctctatgcc 20

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<400> SEQUENCE: 62

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agaagcgctt gattgtcagt a 21

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catcttcatg ctctccgtca g 21

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 <400> SEQUENCE: 67

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 ggtggttggt tgtcagcttc ag 22

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<223> OTHER INFORMATION: TBX6 R primer

<400> SEQUENCE: 74
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<210> SEQ ID NO 75
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<220> FEATURE:
<223> OTHER INFORMATION: TBX10 F primer

<400> SEQUENCE: 75
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<223> OTHER INFORMATION: TBX10 R primer

<400> SEQUENCE: 76
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19

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19

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<223> OTHER INFORMATION: LMO2 F primer

<400> SEQUENCE: 83

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<210> SEQ ID NO 84
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: LMO2 R primer

<400> SEQUENCE: 84

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21

<210> SEQ ID NO 85
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAU F primer

<400> SEQUENCE: 85

cccctggagt tcacgtttca c

21

<210> SEQ ID NO 86
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: TAU R primer

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<400> SEQUENCE: 86

gagagctttg agttgagggga

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What is claimed:

1. An ETS2 treated cell obtained by a method comprising the step of transducing a somatic cell with ETS2 to produce an artificially increased first level of an ETS2 protein in the somatic cell; wherein the artificially increased first level of ETS2 protein transforms the somatic cell into the ETS2 treated cell having surface marker protein levels of SSEA-3 or Tra-1-81 higher than those found in the somatic cell, wherein the somatic cell is a normal human dermal fibroblast cell.

2. The ETS2 treated cell of claim 1, wherein the ETS2 protein comprises the human ETS2 protein sequence of SEQ ID NO:8 encoded on a DNA expression vector transduced in the somatic cell, wherein the human ETS2 protein sequence is expressed in the somatic cell, whereby the first level of the ETS2 protein is artificially increased.

3. The ETS2 treated cell of claim 2, wherein the DNA expression vector comprises the ETS2 DNA coding sequence of SEQ ID NO:9.

4. The ETS2 treated cell of claim 1, wherein an artificially increased first level of an ETS2 protein in the somatic cell transforms the somatic cell into the ETS2 treated cell expressing pluripotent marker genes consisting of NANOG, OCT3/4, SOX2 or c-MYC.

5. The ETS2 treated cell of claim 2, wherein the DNA expression vector comprises a lentiviral vector introduced by infection.

6. A cardiac progenitor cell obtained by a method comprising the step of transducing the ETS2 treated cell of claim 1 with Mesp1 to produce an artificially increased first level of an Mesp1 protein; wherein the artificially increased first level of ETS2 protein and the artificially increased first level of

Mesp1 protein transforms the ETS2 treated cell into the cardiac progenitor cell having levels of contractile protein gene expression of alpha MHC and troponin T higher than those found in the ETS2 treated cell.

7. The cardiac progenitor cell of claim 6, wherein the Mesp1 protein comprises the mouse Mesp1 protein sequence of SEQ ID NO:5 encoded on a DNA expression vector transduced in the ETS2 treated cells, wherein the mouse Mesp1 protein sequence is expressed in the ETS2 treated cells, whereby the first level of the Mesp1 protein is artificially increased.

8. The cardiac progenitor cell of claim 7, wherein the DNA expression vector comprises the Mesp1 DNA coding sequence of SEQ ID NO:6.

9. The cardiac progenitor cell of claim 7, wherein the DNA expression vector comprises a lentiviral vector introduced into the ETS2 treated cells by infection.

10. An induced cardiac progenitor cell obtained by a method comprising the steps of:

(a) transducing a somatic cell with an ETS2 reprogramming factor forming an ETS2 treated cell, wherein the ETS2 treated cell produces a pluripotent stem cell marker protein not present in the somatic cell; and

(b) transducing the ETS2 treated cell with a Mesp1 reprogramming factor forming a cardiac progenitor cell, wherein the cardiac progenitor cell produces a cardiac cell marker protein neither expressed in the somatic cell nor in the ETS2 treated cell;

wherein the somatic cell is a normal human dermal fibroblast cell.

* * * * *