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

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C12N 5/10	(2006.01)
C12N 5/071	(2010.01)
C12N 15/00	(2006.01)
C12N 15/87	(2006.01)
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(52) **U.S. Cl.**

USPC **435/455**; 435/6.13; 435/465; 435/467; 435/476; 435/363; 435/371

(58) **Field of Classification Search**

None

See application file for complete search history.

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(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.

3 Claims, 13 Drawing Sheets

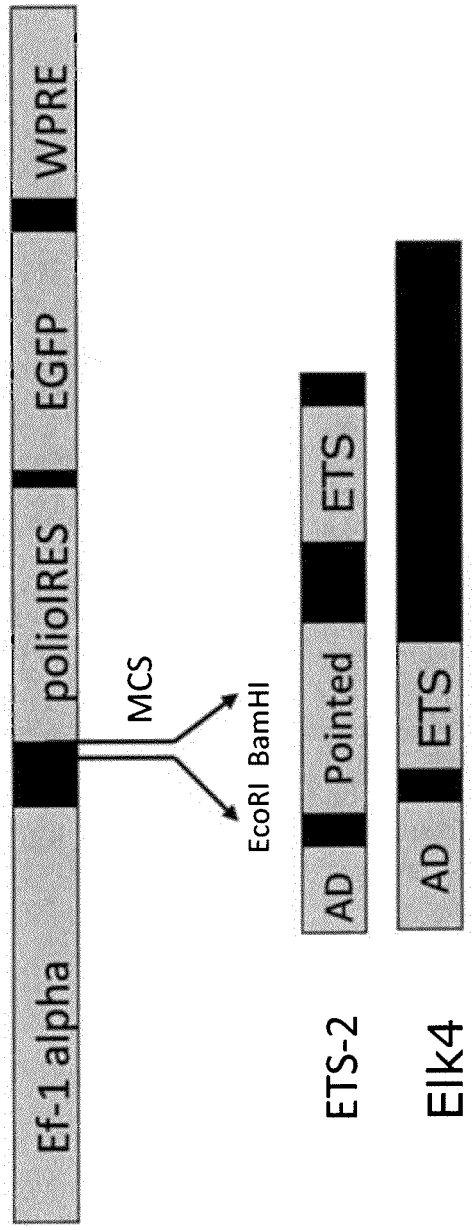

Ef-1 alpha
polioIRES
EGFP
WPRE
MCS
EcoRI BamHI
AD
Pointed
ETS
ETS-2
AD
ETS
Elk4

FIG. 1

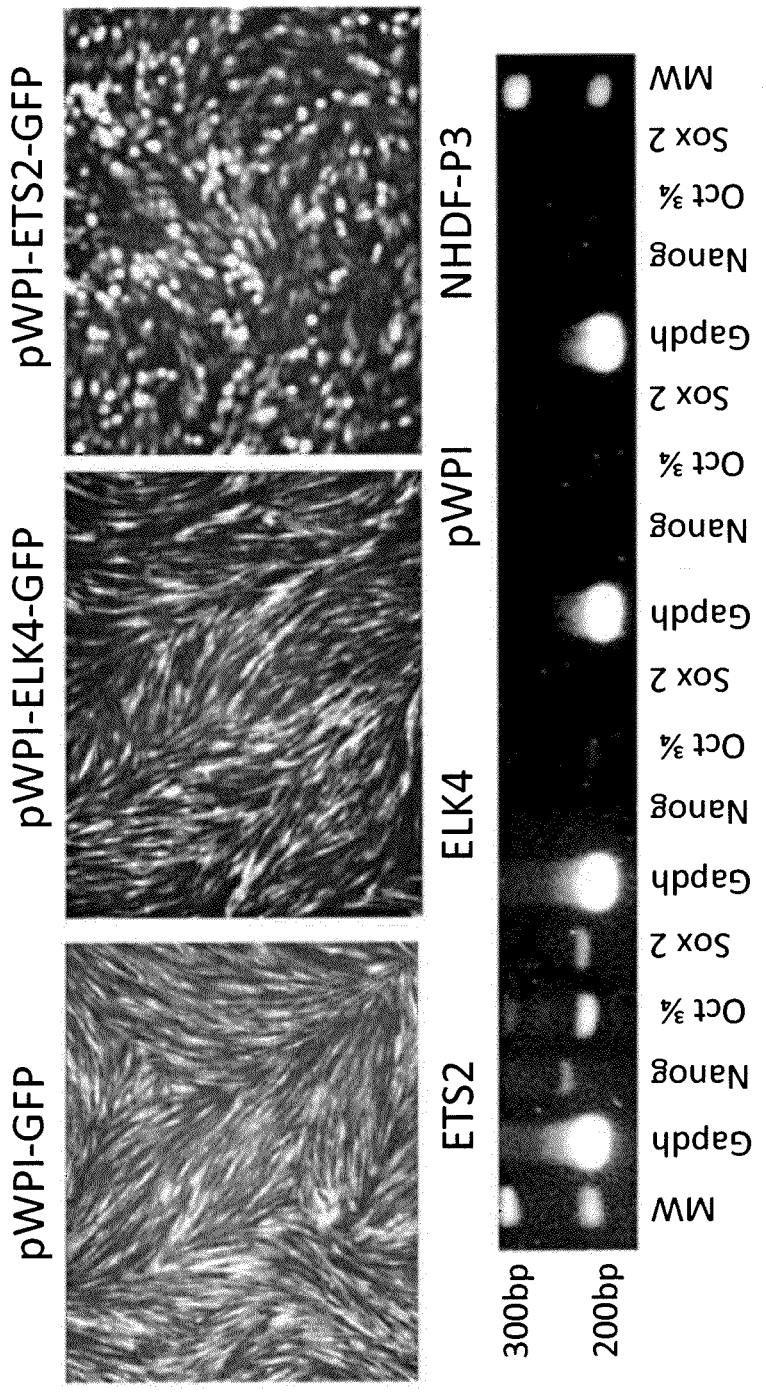

pWPI-GFP
pWPI-ELK4-GFP
pWPI-ETS2-GFP
ETS2
ELK4
pWPI
NHDF-P3
MW
Gapdh
Nanog
Oct ¾
Sox 2
300bp
200bp

FIG. 2

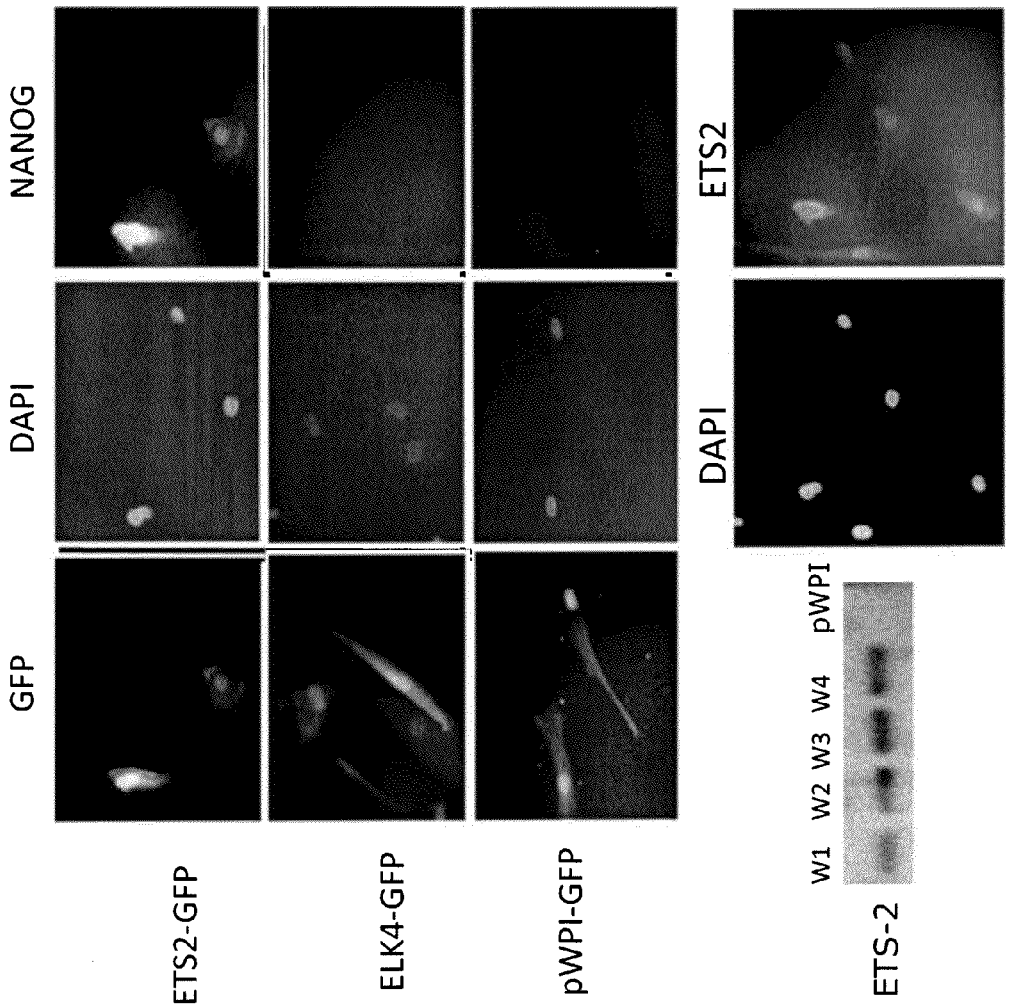

GFP
DAPI
NANOG
ETS2-GFP
ELK4-GFP
pWPI-GFP
ETS2
DAPI
W1 W2 W3 W4 pWPI
ETS-2

FIG. 3

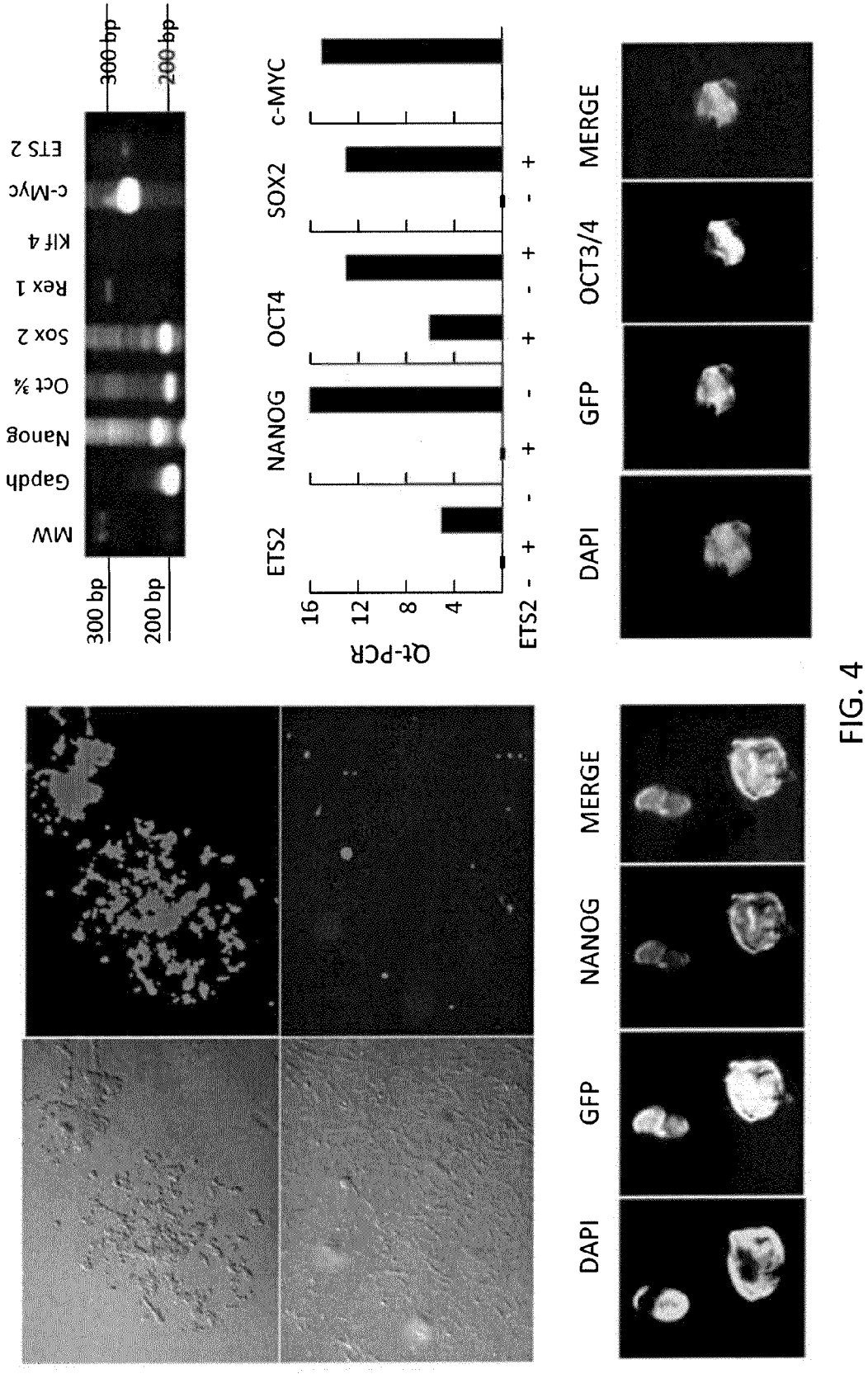

300 bp
200 bp
MW
Gapdh
Nanog
Oct ¾
Sox 2
Rex 1
Klf 4
c-Myc
ETS 2
300 bp
200 bp
Qt-PCR
16
12
8
4
ETS2 - + - + - + - + - +
ETS2
NANOG
OCT4
SOX2
c-MYC
DAPI
GFP
OCT3/4
MERGE
DAPI
GFP
NANOG
MERGE

FIG. 4

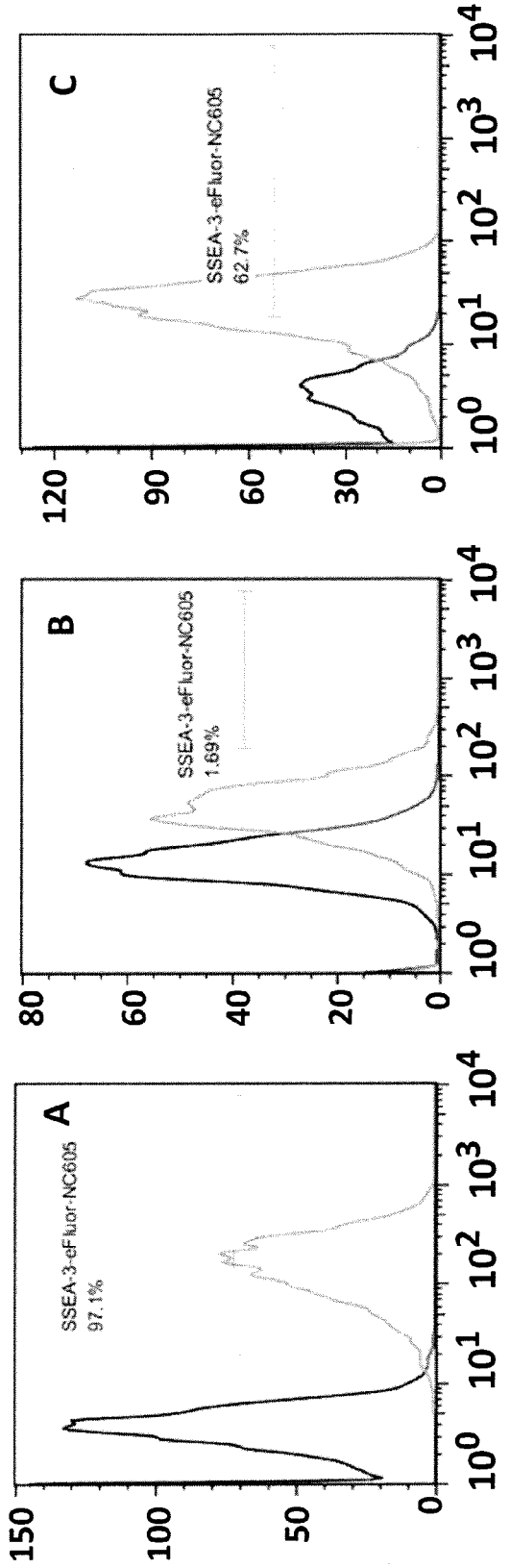

A
SSEA-3-eFluor-NC605
97.1%
B
SSEA-3-eFluor-NC605
1.69%
C
SSEA-3-eFluor-NC605
62.7%

FIG. 5

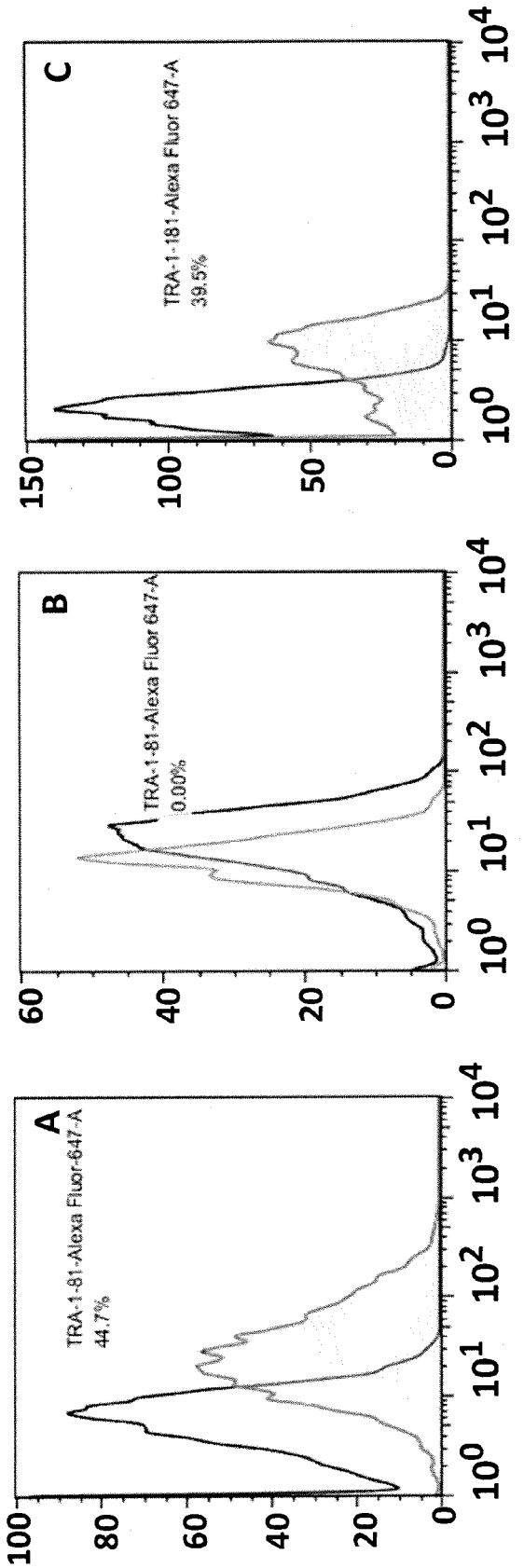

A
TRA-1-81-Alexa Fluor-647-A
44.7%
B
TRA-1-81-Alexa Fluor 647-A
0.00%
C
TRA-1-181-Alexa Fluor 647-A
39.5%

FIG. 6

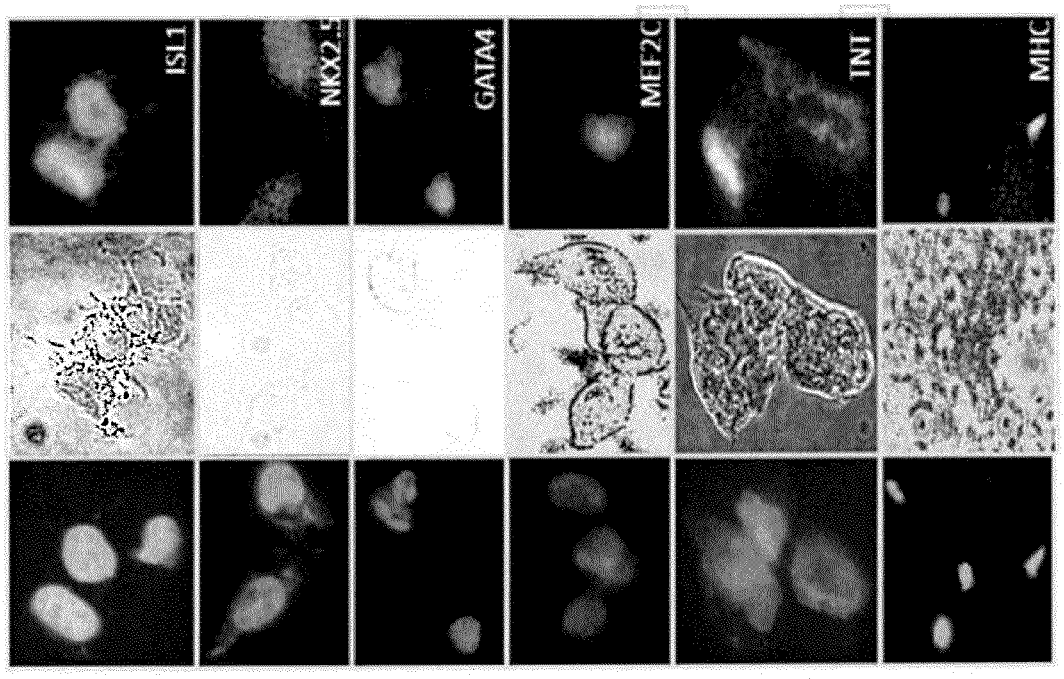


FIG. 7

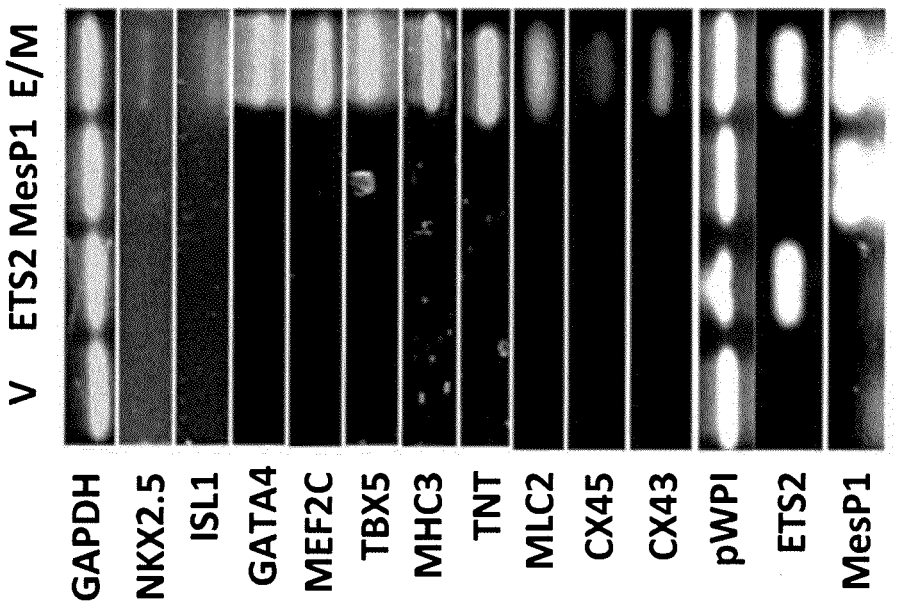

V
ETS2
MesP1
E/M
GAPDH
NKX2.5
ISL1
GATA4
MEF2C
TBX5
MHC3
TNT
MLC2
CX45
CX43
pWPI
ETS2
MesP1

FIG. 8

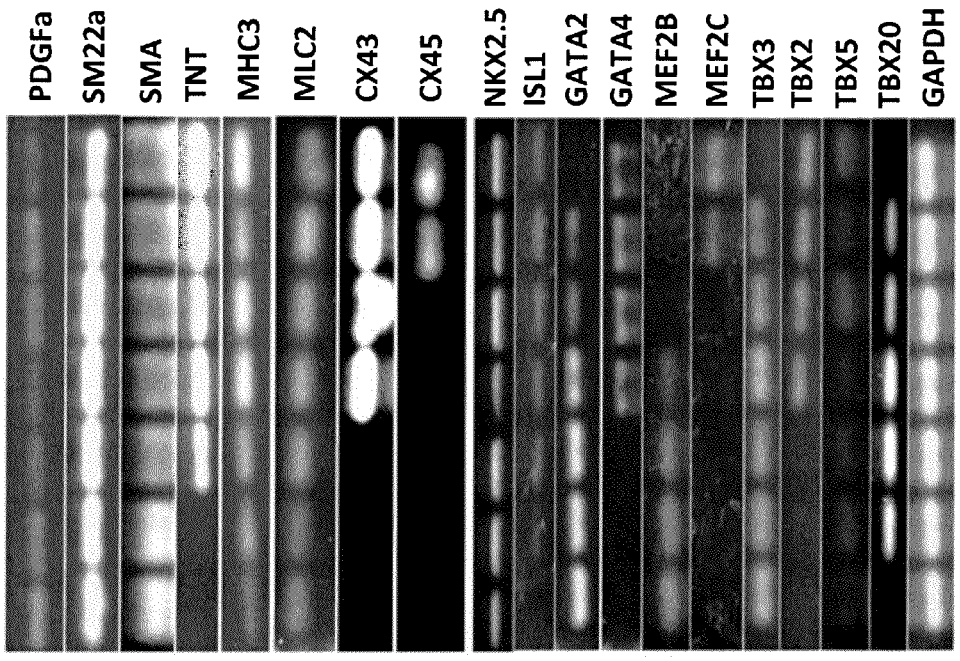
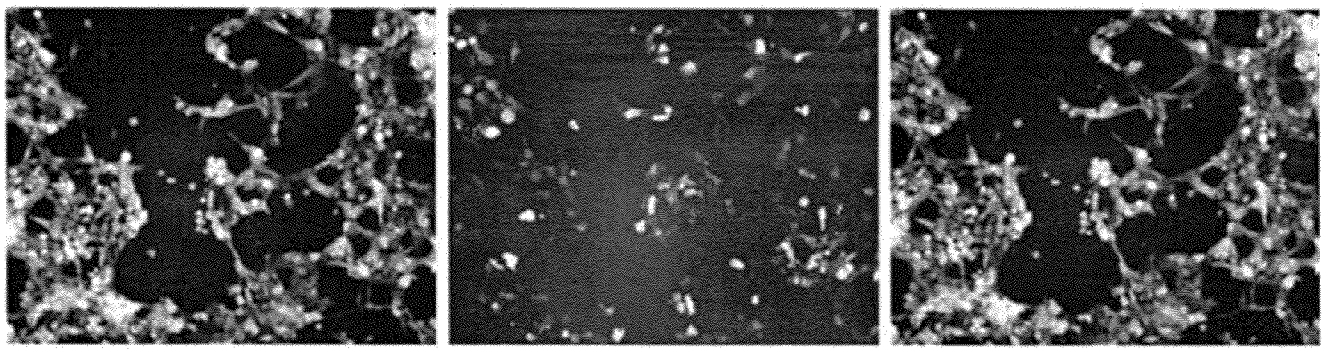

PDGFa
SM22a
SMA
TNT
MHC3
MLC2
CX43
CX45
NKX2.5
ISL1
GATA2
GATA4
MEF2B
MEF2C
TBX3
TBX2
TBX5
TBX20
GAPDH
1 2 3 4 5 6 7 days

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



GFP NKX2.5 Red-Tomato Merge

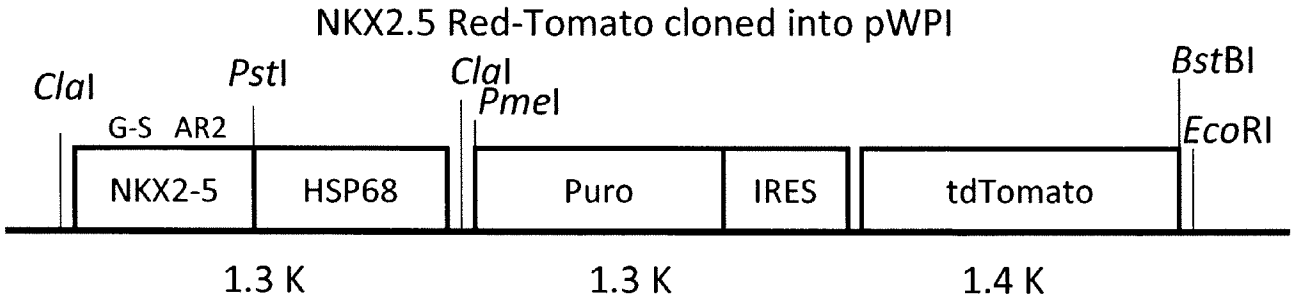


FIG. 10

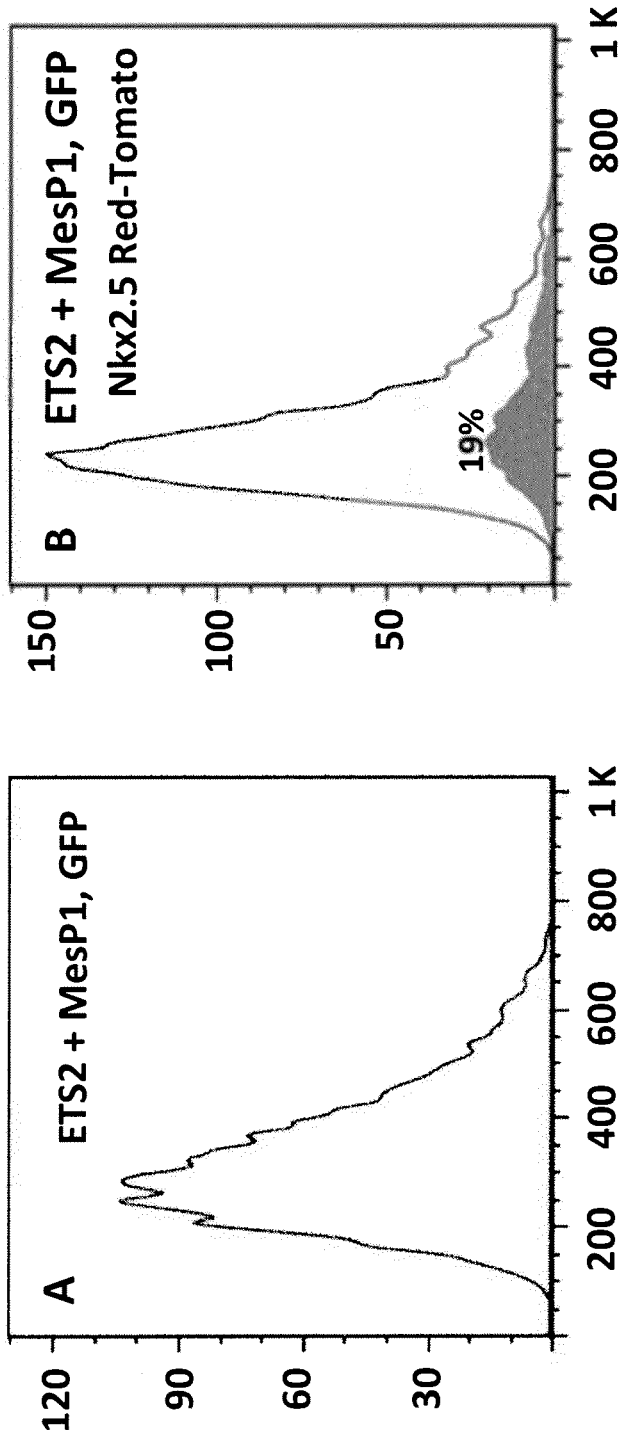

A
ETS2 + MesP1, GFP
120
90
60
30
200
400
600
800
1 K
B
ETS2 + MesP1, GFP
Nkx2.5 Red-Tomato
19%
150
100
50
200
400
600
800
1 K

FIG. 11

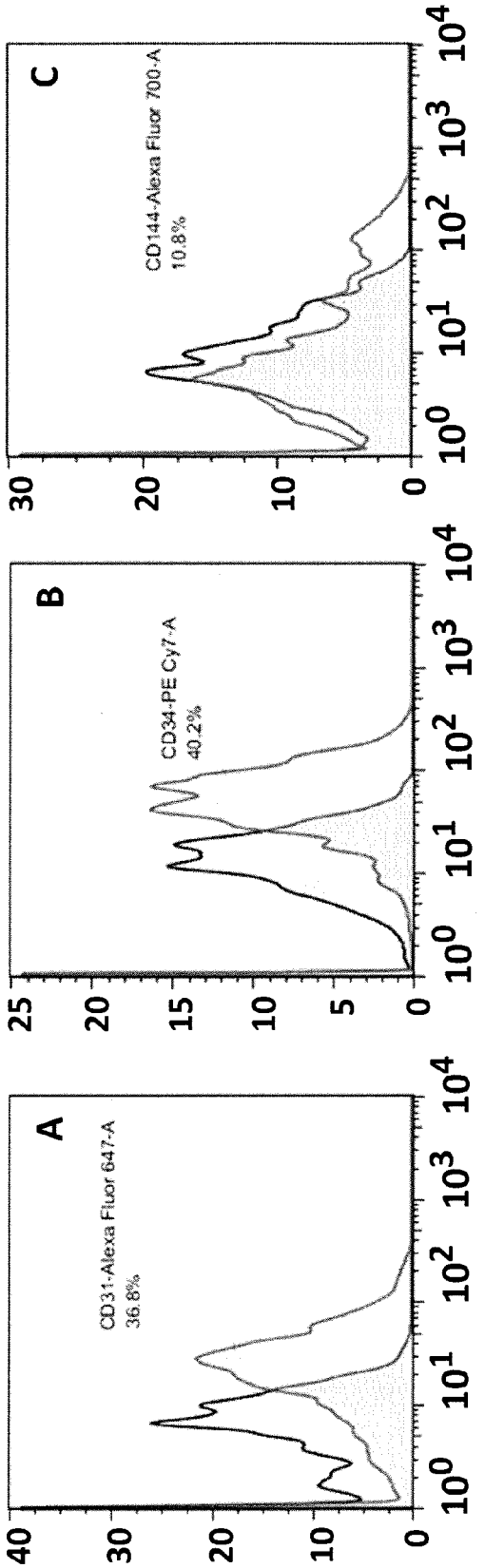

A
CD31-Alexa Fluor 647-A
36.8%
B
CD34-PE Cy7-A
40.2%
C
CD144-Alexa Fluor 700-A
10.8%

FIG. 12

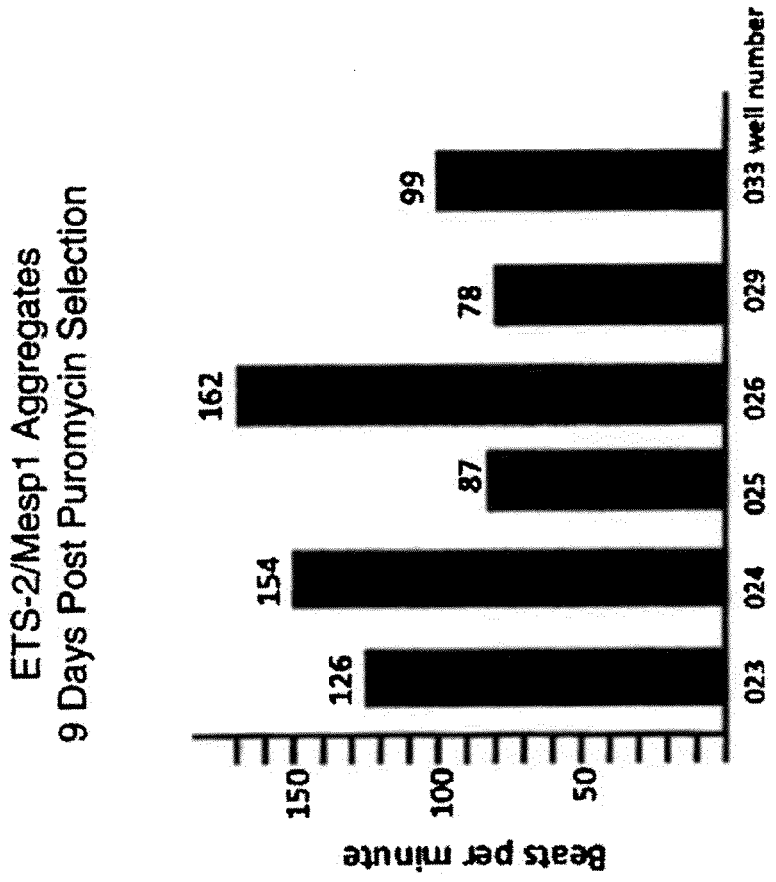

ETS-2/Mesp1 Aggregates
9 Days Post Puromycin Selection
Beats per minute
150
100
50
126
154
87
162
78
99
023
024
025
026
029
033 well number

FIG. 13

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 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-1alpha 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

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 H9 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 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 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.

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 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% CO_2.

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 pWI-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 crtl exp	11	TGTTGCCATCAATGACCCCTT	202
GapdhRP ctrl exp	12	CTCCACGACGTACTCAGCG	
hNanogFP ctrl exp	13	CAGAAGGCCTCAGCACCTAC	225
hNanogRP ctrl exp	14	TATAGAAGGGACTGTTCCAGGC	
hOct $^{3}/_{4}$FP ctrl exp	15	CTTGAATCCCGAATGGAAAGGG	206
hOct $^{3}/_{4}$ RP ctrl exp	16	CCTTCCCAAATAGAACCCCCA	
Sox2 HPB F	17	TGGACAGTTACGCGCACAT	215
Sox2 HPB R	18	CGAGTAGGACATGCTGTAGGT	
hRex1FP ctrl exp	19	GCTGACCACCAGCACACTAGGC	298
hRex1RPctrl exp	20	TTTCTGGTGTCTTGTCTTTGCCCG	

TABLE 1-continued

Primers for Cardiac Progenitor Study

Primer	SEQ ID NO	Seq	Product size
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c-Myc HPB R	22	TTGGACGGACAGGATGTATGC	
hKlf4FP ctrl exp	23	ATGGCTGTCAGCGACGCGCTGCTC	293
hKlf4RP ctrl exp	24	CGTTGAACTCCTCGGTCTCTCTCC	
Nkx 2.5 FP	25	ccctgaccgatcccacctcaac	358
Nkx 2.5 RP	26	GGCGGGCGACGGCGAGATAGC	
Mesp 1 FP	27	tcgaagtggttccttggcagac	162
Mesp 1 RP	28	CCTCCTGCTTGCCTCAAAGTGTC	
Mesp 2 FP	29	CGCTGCGCCTGGCCATCCGCTACAT	113
Mesp 2 RP	30	GCCCCAAGGGGACCCCGCGAC	
Mef2c FP	31	gcaccagtgcagggaacggg	202
Mef2c RP	32	GACTGAGCCGACTGGGAGTTA	
Sox 17 FP	33	gcggcgcaagcaggtgaag	205
Sox 17 RP	34	ACTCTGGCAGTCGCGGTAGTGGC	
FoxA2 FP	35	ctgaagccggaacaccactacgc	214
FoxA2 RP	36	TCCAGGCCCGTTTTGTTCGTGAC	
FGF8 FP	37	agctcagccgccgcctcatccg	313
FGF8 RP	38	AGCCCTCGTACTTGGCATTCTGC	
MyoD FP	39	Aggggctaggttcagctttctcg	240
MyoD RP	40	CTCCTGCTCTGGCAAAGCAACTC	
BMP2 HBP FP	41	ACCTTTATGGAGGGAAACCCA	201
BMP2 HBP RP	42	CCGGATCTGGTTCAAGCATGA	
hTert HBP FP	43	AACCTTCCTCAGCTATGCCC	210
hTert HBP RP	44	GCGTGAAACCTGTACGCCT	
Islet-1 HPB F	45	GTGGAGAGGGCCAGTCTAGG	250
Islet-1 HPB R	46	CCGTCATCTCTACCAGTTGCT	
Troponin T HPB F	47	GAGTTGCAGGCGCTGATTG	229
Troponin T HPB R	48	TCTGGATGTAACCCCCAAAATG	
Dhand HPB F	49	ATGAGTCTGGTAGGTGGTTTTCC	205
Dhand HPB R	50	CATACTCGGGGCTGTAGGACA	
T HPB F	51	GATCACGCAGCTCAAGATTGC	230
T HPB R	52	TCTCTGGTGTGTTCCTAGACG	
T5 HPB F	53	CACTTCTCCGCTCACTTCACC	210
T5 HPB R	54	TGGCACGCCATGAGAGTAGA	
ETS-2 HPB F	55	AAAGCTACCTTCAGTGGCTTC	225
ETS-2 HPB R	56	AATGTCACCCACAAAGTCAGG	
Dkk-1 FP	57	ATTCCAACGCTATCAAGAACC	384
Dkk-1 RP	58	CCAAGGTGCTATGATCATTACC	
TBX 20 FP	59	tccagattctccttttaccg	190
TBX 20 RP	60	ttcagacttcaggttgagca	
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SM actin HBP R	62	CACGCTCAGTCAGGATCTTCA	
hGATA1 F	63	AGAAGCGCCTGATTGTCAGTA	229
hGATA1 R	64	AGAGACTTGGGTTGTCCAGAA	
hGTAT2 F	65	GGCCCACTCTCTGTGTACC	243
hGATA2 R	66	CATCTTCATGCTCTCCGTCAG	
TBX3-1 F	67	GTGTCTCGGGCCTGGATTC	164
TBX3-1 R	68	ACGTGTAGGGGTAAGGGAACA	
TBX3-2 F	69	TTAAAGTGAGATGTTCTGGGCTG	298
TBX3-2 R	70	ACTATAATTCCCCTGCCACGTA	
TBX4 F	71	TGACCATCGCTACAAGTTCTGT	163
TBX4 R	72	GGTGGTTGTTTGTCAGCTTCAG	
TBX6 F	73	ACACCCCTAAACTGGATTGCT	229
TBX6 R	74	CCTCCCAGCTTTGGTGATGAT	
TBX10 F	75	CCTCGGCATACTTGCACCC	208
TBX10 R	76	ATTCCTCCCACAGAGGCTTCA	
TBX18 F	77	GCCCCTGCTGACTATTCTGC	227
TBX18 R	78	CTGCATGGATAAGCTGGTCTG	
TBX19 F	79	AAGAATGGCAGACGGATGTTT	204
TBX19 R	80	CCGGGTGAATGTAGACGCAG	
RUNX2 F	81	CGGCAAAATGAGCGACGTG	268
RunX2 R	82	CACCGAGCACAGGAAGTTG	
LMO2 F	83	GGCCATCGAAAGGAAGAGCC	221
LMO2 R	84	GGCCCAGTTTGTAGTAGAGGC	
TAU F	85	CCCCTGGAGTTCACGTTTCAC	240
TAU R	86	GCGAGCTTTGAGTTGAGGGA	

Example 4

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 HNDF-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, TNT 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, TNT, 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 lentivirus, 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 prometer 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.

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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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aagcacatga tctgttggac ctctaatgat gggcagttta agcttttgca ggcagaagag    120

gtggctcgtc tctgggggat tcgcaagaac aagcctaaca tgaattatga caaactcagc    180

cgagccctca gatactatta tgtaaagaat atcatcaaaa aagtgaatgg tcagaagttt    240

gtgtacaagt ttgtctctta tccagagatt ttgaacatgg atccaatgac agtgggcagg    300

attgagggtg actgtgaaag tttaaacttc agtgaagtca gcagcagttc caaagatgtg    360
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gagaatggag ggaaagataa accacctcag cctggtgcca agacctctag ccgcaatgac 420

tacatacact ctggcttata ttcttcattt actctcaact ctttgaactc ctccaatgta 480

aagcttttca aattgataaa gactgagaat ccagccgaga aactggcaga gaaaaaatct 540

cctcaggagc ccacaccatc tgtcatcaaa tttgtcacga caccttccaa aaagccaccg 600

gttgaacctg ttgctgccac catttcaatt ggcccaagta tttctccatc ttcagaagaa 660

actatccaag ctttggagac attggtttcc ccaaaactgc cttccctgga agccccaacc 720

tctgcctcta acgtaatgac tgcttttgcc accacaccac ccatttcgtc catacccccc 780

ttgcaggaac ctcccagaac accttcacca ccactgagtt ctcacccaga catcgacaca 840

gacattgatt cagtggcttc tcagccaatg gaacttccag agaatttgtc actggagcct 900

aaagaccagg attcagtctt gctagaaaag gacaaagtaa ataattcatc aagatccaag 960

aaacccaaag ggttagaact ggcacccacc cttgtgatca cgagcagtga tccaagccca 1020

ctgggaatac tgagcccatc tctccctaca gcttctctta caccagcatt tttttcacag 1080

gtagcttgct cgctctttat ggtgtcacca ttgctttcat ttatttgccc ttttaagcaa 1140

atccagaatt tatacactca agtttgcttt ctgttactta ggtttgtctt agaaaggtta 1200

tgtgtgactg tcatgtga 1218

<210> SEQ ID NO 4
<211> LENGTH: 1122
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: mouse MesP1 gene sequence

<400> SEQUENCE: 4

gatatcgaat tctggaaggg gcccgcttca cacctagggc tcaggataaa gctacagcgg 60

acccaatggt caggcctccg ttgccatggc ccagcccctg tgcgagccgc gctccgagtc 120

ctggatcctg agtcccgctg gtcggcagcc accgatgcct tccgatggga acagcgtctg 180

ctccccagcc tggtcctcgg acccgtggga cggtgcccag gccagcagcc ctgcaccacc 240

ctgcgcccgc ccggcccggc gtgctgggac cccgggtagg cgcgggacgc acggtagccg 300

cctgggtagc ggacagcggc agagcgccag cgagcgggag aagctacgta tgcgcacact 360

cgcccgcgcg ctgcacgagc tgcgccgctt cttgccgcca tccgtggcac caaccggcca 420

gaacctgacc aagatcgaga cgctgcgcct ggccatccgc tacattggcc acctgtcggc 480

tgtgctggga ctcagcgagg acaacctccg gcgacagcgg cacgcggtgt cacctcgagg 540

ctgcccgctg tgccccgaca gcgacctggc gcagtcgcag tcactcggtc ctggtttaag 600

cccggccgtc tgcagcgggg tgtcgtgggg atccccgcct gcctacccta gaccccgagt 660

cgccgcagaa tcgtgggacc catcgttcca gtacgcagaa acagcatccc aggaaaggca 720

ggaaatggag cccagtccct catctccgct cttcagcagc gacatgctgg ctcttctaga 780

aacctggacg ccgccgcagg agtggccgcc tgcctgaaga gtggagggga caatgcaacg 840

gatgattgtc accctgtctg agcacagaca cttttccttt ggtcttggca ccttcggagg 900

gagtagatcc tggaagaggc ggcagtgata ccaacatggg catcccgggg tgggagctgg 960

ccctcatcca gactgtacca ttccaaccct ccttggaagg aggcgcccaa tagggtacac 1020

gctctaaaga tgaagcaggc 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

<400> SEQUENCE: 5

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

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

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cagccaccga tgccttccga tgggaacagc gtctgctccc cagcctggtc ctcggacccg 120

tgggacggtg cccaggccag cagccctgca ccaccctgcg cccgcccggc ccggcgtgct 180

gggaccccgg gtaggcgcgg gacgcacggt agccgcctgg gtagcggaca gcggcagagc 240

gccagcgagc gggagaagct acgtatgcgc acactcgccc gcgcgctgca cgagctgcgc 300

cgcttcttgc cgccatccgt ggcaccaacc ggccagaacc tgaccaagat cgagacgctg 360

cgcctggcca tccgctacat tggccacctg tcggctgtgc tgggactcag cgaggacaac 420

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ctccggcgac agcggcacgc ggtgtcacct cgaggctgcc cgctgtgccc cgacagcgac 480

ctggcgcagt cgcagtcact cggtcctggt ttaagcccgg ccgtctgcag cggggtgtcg 540

tggggatccc cgcctgccta ccctagaccc cgagtcgccg cagaatcgtg ggacccatcg 600

ttccagtacg cagaaacagc atcccaggaa aggcaggaaa tggagcccag tccctcatct 660

ccgctcttca gcagcgacat gctggctctt ctagaaacct ggacgccgcc gcaggagtgg 720

ccgcctgcct ga 732

<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

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ctctggccgg cgccctcgcc ctcgcccggc gcgcaccgag cagccgcggg cgccgagcag 120

ccaccgtccc gaccaagcgc cggccctgcc cgcagcggca ggatgaatga tttcggaatc 180

aagaatatgg accaggtagc ccctgtggct aacagttaca gagggacact caagcgccag 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 caaagaaccc ctggctgtgg 480

agtgagcaac aggtatgcca gtggcttctc tgggccacca atgagttcag tctggtgaac 540

gtgaatctgc agaggttcgg catgaatggc cagatgctgt gtaaccttgg caaggaacgc 600

tttctggagc tggcacctga ctttgtgggt gacattctct gggaacatct ggagcaaatg 660

atcaaagaaa accaagaaaa gacagaagat caatatgaag aaaattcaca cctcacctcc 720

gttcctcatt ggattaacag caatacatta ggttttggca cagagcaggc gccctatgga 780

atgcagacac agaattaccc caaaggcggc ctcctggaca gcatgtgtcc ggcctccaca 840

cccagcgtac tcagctctga gcaggagttt cagatgttcc ccaagtctcg gctcagctcc 900

gtcagcgtca cctactgctc tgtcagtcag gacttcccag gcagcaactt gaatttgctc 960

accaacaatt ctgggacgcc caaagaccac gactcccctg agaacggtgc ggacagcttc 1020

gagagctcag actccctcct ccagtcctgg aacagccagt cgtccttgct ggatgtgcaa 1080

cgggttcctt ccttcgagag cttcgaagat gactgcagcc agtctctctg cctcaataag 1140

ccaaccatgt ctttcaagga ttacatccaa gagaggagtg acccggtgga gcaaggcaaa 1200

ccagttatac ctgcagctgt gctggccggc ttcacaggaa gtggacctat tcagctgtgg 1260

cagtttctcc tggagctgct atcagacaaa tcctgccagt cattcatcag ctggactgga 1320

gacggatggg agtttaagct cgccgacccc gatgaggtgg cccgccggtg gggaaagagg 1380

aaaaataagc ccaagatgaa ctacgagaag ctgagccggg gcttacgcta ctattacgac 1440

aagaacatca tccacaagac gtcggggaag cgctacgtgt accgcttcgt gtgcgacctc 1500

cagaacttgc tggggttcac gcccgaggaa ctgcacgcca tcctgggcgt ccagcccgac 1560

acggaggact gaggtcgccg ggaccaccct gagccggccc caggctcgtg gactgagtgg 1620

gaagcccatc ctgaccagct gctccgagga cccaggaaag gcaggattga aaatgtccag 1680

gaaagtggcc aagaagcagt ggccttattg catcccaaac cacgcctctt gaccaggctg 1740

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cctcccttgt ggcagcaacg gcacagctaa ttctactcac agtgctttta agtgaaaatg 1800

gtcgagaaag aggcaccagg aagccgtcct ggcgcctggc agtccgtggg acgggatggt 1860

tctggctgtt tgagattctc aaaggagcga gcatgtcgtg gacacacaca gactattttt 1920

agattttctt ttgccttttg caaccaggaa cagcaaatgc aaaaactctt tgagagggta 1980

ggagggtggg aaggaaacaa ccatgtcatt tcagaagtta gtttgtatat attattataa 2040

tcttataatt gttctcagaa tcccttaaca gttgtattta acagaaattg tatattgtaa 2100

tttaaaataa ttatataact gtatttgaaa taagaattca gacatctgag gttttatttc 2160

atttttcaat agcacatatg gaattttgca aagatttaat ctgccaaggg ccgactaaga 2220

gaagttgtaa agtatgtatt atttacattt aatagactta cagggataag gcctgtgggg 2280

ggtaatccct gctttttgtg ttttttgtt tgtttgtttg tttgtttttg gggggttttc 2340

ttgccttggt tgtctggcaa ggactttgta catttgggag tttttatgag aaacttaaat 2400

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taaagcagta tttttcttga caaaaaaaaa aaaaaaaaaa 2500

<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

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

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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
    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                 395                 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
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<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 ggtccctgtt tgctgttttt    120

ccttctctaa atgaagagca aacactgcaa gaagtgccaa caggcttgga ttccatttct    180

catgactccg ccaactgtga attgcctttg ttaaccccgt gcagcaaggc tgtgatgagt    240

caagccttaa aagctacctt cagtggcttc aaaaaggaac agcggcgcct gggcattcca    300

aagaacccct ggctgtggag tgagcaacag gtatgccagt ggcttctctg ggccaccaat    360

gagttcagtc tggtgaacgt gaatctgcag aggttcggca tgaatggcca gatgctgtgt    420

aaccttggca aggaacgctt tctggagctg gcacctgact ttgtgggtga cattctctgg    480

gaacatctgg agcaaatgat caaagaaaac caagaaaaga cagaagatca atatgaagaa    540

aattcacacc tcacctccgt tcctcattgg attaacagca atacattagg ttttggcaca    600

gagcaggcgc cctatggaat gcagacacag aattacccca aaggcggcct cctggacagc    660

atgtgtccgg cctccacacc cagcgtactc agctctgagc aggagtttca gatgttcccc    720
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aagtctcggc tcagctccgt cagcgtcacc tactgctctg tcagtcagga cttcccaggc 780

agcaacttga atttgctcac caacaattct gggacgccca aagaccacga ctcccctgag 840

aacggtgcgg acagcttcga gagctcagac tccctcctcc agtcctggaa cagccagtcg 900

tccttgctgg atgtgcaacg ggttccttcc ttcgagagct tcgaagatga ctgcagccag 960

tctctctgcc tcaataagcc aaccatgtct ttcaaggatt acatccaaga gaggagtgac 1020

ccggtggagc aaggcaaacc agttatacct gcagctgtgc tggccggctt cacaggaagt 1080

ggacctattc agctgtggca gtttctcctg gagctgctat cagacaaatc ctgccagtca 1140

ttcatcagct ggactggaga cggatgggag tttaagctcg ccgaccccga tgaggtggcc 1200

cgccggtggg gaaagaggaa aaataagccc aagatgaact acgagaagct gagccggggc 1260

ttacgctact attacgacaa gaacatcatc cacaagacgt cggggaagcg ctacgtgtac 1320

cgcttcgtgt gcgacctcca gaacttgctg gggttcacgc ccgaggaact gcacgccatc 1380

ctgggcgtcc agcccgacac ggaggactga 1410

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

<400> SEQUENCE: 10

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tagtaagtga ggtgctgaga gcagaatgag ctactttgtg gattctgctg ggagcagccc 120

cgtcccttac tcagcgcctc gtcctgcagt ggtgaggcaa ggacctagca acacttatga 180

agatcctcga atgaactgtg gtttccagtc caattatcac cagcaaagac cttgctaccc 240

cttttgggat gagatggcaa ctcaggaagt tcctactggt cttgaacact gtgtctcaga 300

tatggaatgt gcagatgtcc cactattaac tccaagcagc aaagaaatga tgtctcaagc 360

attaaaagct actttcagtg gtttcactaa agaacagcaa cgactgggga tcccaaaaga 420

cccccggcag tggacagaaa cccatgttcg ggactgggtg atgtgggctg tgaatgaatt 480

cagcctgaaa ggtgtagact tccagaagtt ctgtatgaat ggagcagccc tctgcgccct 540

gggtaaagac tgctttctcg agctggcccc agactttgtt ggggacatct tatgggaaca 600

tctagagatc ctgcagaaag aggatgtgaa accatatcaa gttaatggag tcaacccagc 660

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gtgtgttcca ccatcggagt tctcagagcc cagcttcatc acagagtcct atcagacgct 780

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cattctccga gaccctctcc agacagacac cttgcagaat gactactttg ctatcaaaca 900

agaagtcgtc accccagaca acatgtgcat ggggaggacc agtcgtggta aactcggggg 960

ccaggactct tttgaaagca tagagagcta cgatagttgt gatcgcctca cccagtcctg 1020

gagcagccag tcatctttca acagcctgca gcgtgttccc tcctatgaca gcttcgactc 1080

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agatgaggtg gccaggagat ggggaaagag gaaaaacaaa cctaagatga attatgagaa 1380

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gctgcacgcc atgctggacg tcaagccaga tgccgacgag tgatggcact gaaggggctg 1560

gggaaaccct gctgagacct tccaaggaca gccgtgttgg ttggactctg aattttgaat 1620

tgttattcta ttttttattt tccagaactc atttttttacc ttcaggggtg ggagctaagt 1680

cagttgcagc tgtaatcaat tgtgcgcagt tgggaaagga aagccaggac ttgtggggtg 1740

ggtgggacca gaaattcttg agcaaatttt caggagaggg agaagggcct tctcagaagc 1800

ttgaaggctc tggcttaaca gagaaagaga ctaatgtgtc caatcatttt taaaaatcat 1860

ccatgaaaaa gtgtcttgag ttgtggaccc attagcaagt gacattgtca catcagaact 1920

catgaaactg atgtaaggca attaatttgc ttctgttttt aggtctggga gggcaaaaaa 1980

gaggtgggtg ggatgaaaca tgttttgggg ggggatgcac tgaaaatctg agaactattt 2040

acctatcact ctagttttga agcaaagatg gacttcagtg gggaggggcc aaaaccgttg 2100

ttgtgttaaa atttatttta ttaaattttg tgccagtatt ttttttctta aaaatcgtct 2160

taagctctaa ggtggtctca gtattgcaat atcatgtaag tttgttttta tttgccggct 2220

gaggattctg tcacaatgaa agaaaactgt ttatatagac cccattggaa aagcaaaacg 2280

ctctcactga gatcagggat cccaaattca tgggacttat ataagaagga caattaatgc 2340

tgatttgggt acaggggaat tatgtgtgtg aatgtcatct acaattaaaa aaaattagca 2400

catcccttta cttacttgtt atcagtggat tctcggggtt tggacttaat gttgagctaa 2460

gaagcattaa gtctttgaac tgaatgtatt ttgcatccct ggttttggac gacagtaaac 2520

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tcttagtcct atatctgtag catagatgac ttggaataaa agctgtatgc atgggcatta 2640

cccctcaggt cctaagaaat aagtcctgaa tgcatgtcgt tccaaactaa cactctgtaa 2700

tttttctttt atgtcttatt ttccaagagt cctccatttt ttgcaccccc tcaccgccaa 2760

ctctgttatt cagtagagag aagtgtacgg ctttctgatt ggtgagtgaa aaagtaactt 2820

gagacacgac ctaagttgaa gagtttagac ttgctgagtt ttagaagtga tggaaattaa 2880

gagagcattt caataaaatg tgacttggct gtctttggaa gagaagtgca aggctttcct 2940

ttgaagaatt taaattagtc cggtaggatg tcaggtgaga ctgtgtatgc aaaatgaatg 3000

gcacaggtga tgccagggcc tcttgcttgg gtctgatgtc ttggcacagg gtaagtgaag 3060

gttaattcca gaagagagga atgacttgaa ggcaaaggaa actaaggaag gaggttcagt 3120

gaggaaaata aggttgtcca tgagatttga atagattttt agttccccca aggtttaaat 3180

acaaacatag tcaagcaagg tagtcatctt tctgctggtt gtgaggggga atctgaaaat 3240

ggagttttag aggaaaagtc aacatctaac tagtgaggaa aagtgcctaa tacaattaga 3300

atctccctca ctctatagtt gcccagttga aaggataagg aggaggggtg gcttttatgg 3360

acttccatga gagaaggaaa gaaatatttc aggtaagctt ctcagggctg gccctttttg 3420

ggatttggat gagaaattgg aagtactaac tactttctag catatcttta agaaaattga 3480

ttgttattta ctcccagatc ctcttgcaga cccagaatta tcaggaacat agctctgtga 3540

ttcatgagtg tccccatact gatgaattgg agcatccata tggaaagcaa aggcagaatt 3600

atcccagctg tattattttg atcttttgga tgcaggtgcc ttaatgaagc tctcaaaata 3660

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aaaatgcaaa aaaattctta tggcggagtt ttttgtttgt ttatttttagt agctgatgct 5040

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<400> SEQUENCE: 11

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<223> OTHER INFORMATION: GapdhRP ctrl exp primer

<400> SEQUENCE: 12

ctccacgacg tactcagcg 19

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cagaaggcct cagcacctac 20

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<400> SEQUENCE: 14

tatagaaggg actgttccag gc 22

<210> SEQ ID NO 15
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<223> OTHER INFORMATION: Sox2 HPB F primer

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tggacagtta cgcgcacat 19

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<223> OTHER INFORMATION: Sox2 HPB R primer

<400> SEQUENCE: 18

cgagtaggac atgctgtagg t 21

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gctgaccacc agcacactag gc 22

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<223> OTHER INFORMATION: hRex1RPctrl exp primer

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<223> OTHER INFORMATION: hKlf4FP ctrl exp primer

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<210> SEQ ID NO 27

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<400> SEQUENCE: 28

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<220> FEATURE:
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<223> OTHER INFORMATION: Mesp 2 RP primer

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<210> SEQ ID NO 31
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<400> SEQUENCE: 31

gcaccagtgc agggaacggg 20

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<223> OTHER INFORMATION: Mef2c RP primer

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<223> OTHER INFORMATION: Sox 17 FP primer

<400> SEQUENCE: 33

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gcggcgcaag caggtgaag 19

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<400> SEQUENCE: 34

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<210> SEQ ID NO 35
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<212> TYPE: DNA
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<400> SEQUENCE: 35

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<400> SEQUENCE: 37

agctcagccg ccgcctcatc cg 22

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<223> OTHER INFORMATION: FGF8 RP primer

<400> SEQUENCE: 38

agccctcgta cttggcattc tgc 23

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<400> SEQUENCE: 39

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<223> OTHER INFORMATION: MyoD RP primer

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<223> OTHER INFORMATION: BMP2 HBP FP primer

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<223> OTHER INFORMATION: hTert HBP FP primer

<400> SEQUENCE: 43

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<223> OTHER INFORMATION: hTert HBP RP primer

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gcgtgaaacc tgtacgcct 19

<210> SEQ ID NO 45
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Islet-1 HPB F primer

<400> SEQUENCE: 45

gtggagaggg ccagtctagg 20

<210> SEQ ID NO 46
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Islet-1 HPB R primer

<400> SEQUENCE: 46

ccgtcatctc taccagttgc t 21

<210> SEQ ID NO 47

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<211> LENGTH: 19
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<400> SEQUENCE: 47

gagttgcagg cgctgattg 19

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<212> TYPE: DNA
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<223> OTHER INFORMATION: Troponin T HPB R primer

<400> SEQUENCE: 48

tctggatgta acccccaaaa tg 22

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<223> OTHER INFORMATION: Dhand HPB F primer

<400> SEQUENCE: 49

atgagtctgg taggtggttt tcc 23

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<223> OTHER INFORMATION: Dhand HPB R primer

<400> SEQUENCE: 50

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<223> OTHER INFORMATION: T HPB F primer

<400> SEQUENCE: 51

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<223> OTHER INFORMATION: T HPB R primer

<400> SEQUENCE: 52

tctctggtgt gttcctagac g 21

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<223> OTHER INFORMATION: T5 HPB F primer

<400> SEQUENCE: 53

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cacttctccg ctcacttcac c 21

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attccaacgc tatcaagaac c 21

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<223> OTHER INFORMATION: Dkk-1 RP primer

<400> SEQUENCE: 58

ccaaggtgct atgatcatta cc 22

<210> SEQ ID NO 59
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<212> TYPE: DNA
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<223> OTHER INFORMATION: TBX 20 FP primer

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<212> TYPE: DNA
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<223> OTHER INFORMATION: TBX 20 RP primer

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<223> OTHER INFORMATION: SM actin HBP F primer

<400> SEQUENCE: 61

cggtgctgtc tctctatgcc 20

<210> SEQ ID NO 62
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<223> OTHER INFORMATION: SM actin HBP R primer

<400> SEQUENCE: 62

cacgctcagt caggatcttc a 21

<210> SEQ ID NO 63
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<223> OTHER INFORMATION: hGATA1 F primer

<400> SEQUENCE: 63

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<223> OTHER INFORMATION: hGATA1 R primer

<400> SEQUENCE: 64

agagacttgg gttgtccaga a 21

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<223> OTHER INFORMATION: hGTAT2 F primer

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ggcccactct ctgtgtacc 19

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catcttcatg ctctccgtca g 21

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gtgtctcggg cctggattc 19

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<220> FEATURE:
<223> OTHER INFORMATION: TBX3-2 R primer

<400> SEQUENCE: 70

actataattc ccctgccacg ta 22

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<223> OTHER INFORMATION: TBX4 F primer

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tgaccatcgc tacaagttct gt 22

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ggtggttgtt tgtcagcttc ag 22

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<223> OTHER INFORMATION: TBX6 F primer

<400> SEQUENCE: 73

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acacccctaa actggattgc t 21

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: TBX6 R primer

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cctcccagct ttggtgatga t 21

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<223> OTHER INFORMATION: TBX10 F primer

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<223> OTHER INFORMATION: TBX10 R primer

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<220> FEATURE:
<223> OTHER INFORMATION: TBX18 F primer

<400> SEQUENCE: 77

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<400> SEQUENCE: 78

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<210> SEQ ID NO 79
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<223> OTHER INFORMATION: TBX19 F primer

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aagaatggca gacggatgtt t 21

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<223> OTHER INFORMATION: TBX19 R primer

<400> SEQUENCE: 80

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<223> OTHER INFORMATION: RunX2 R primer

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<210> SEQ ID NO 83
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LMO2 F primer

<400> SEQUENCE: 83

ggccatcgaa aggaagagcc 20

<210> SEQ ID NO 84
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LMO2 R primer

<400> SEQUENCE: 84

ggcccagttt gtagtagagg c 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
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: TAU R primer

<400> SEQUENCE: 86

gcgagctttg agttgaggga 20

What is claimed:

1. A method for preparing a cardiac progenitor cell, the method comprising:

(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.

2. The method of claim **1**, wherein the ETS2 comprises the DNA sequence having SEQ ID NO:9.

3. The method of claim **1**, wherein the Mespl comprises the DNA sequence having SEQ ID NO:6.

* * * * *