Epigenetic regulation on the efficiency of nuclear reprogramming

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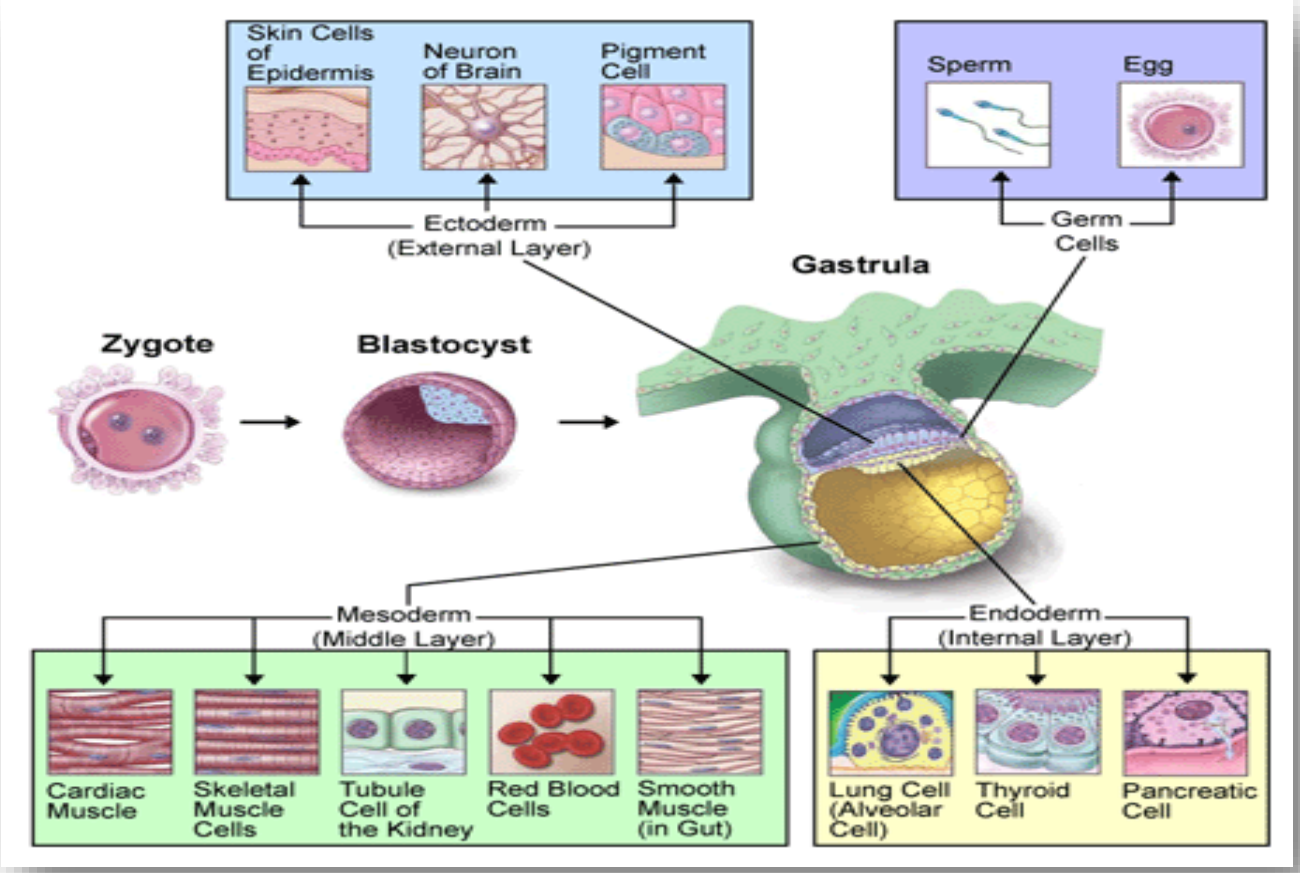
이 동 율

Embryonic Stem Cells

Pluripotency & un-limited proliferation



Cells in the inner cell mass are pluripotent and can develop into all kinds of organs

Thomson et al., Science 1998

Specialty of Embryonic Stem Cells

1. ***Pluripotency (Teratoma formation)***
2. ***Un-limited proliferation***
3. ***High genetic stability during long-term culture***
4. ***Ethical problem***
5. ***Immune recognition***

➔ ***Required Patient-Specific Pluripotent Stem Cells***

For the Clinical Application of Pluripotent Stem Cells

The strategies for preventing hESC immune recognition **:**

- Induction of tolerance by hematopoietic chimerism using hematopoietic cells differentiated from hESCs
- Creation of universal cells by genetic modification to reduce the expression of MHC molecule
- **Establishment of large banks of immunophenotyped hESC lines** to match MHC alleles between hESC lines and patients

or

Generation of Isogenic Plutripotent Stem Cell Lines *using the patient's own somatic cells by* ***SCNT or IPS technologies***

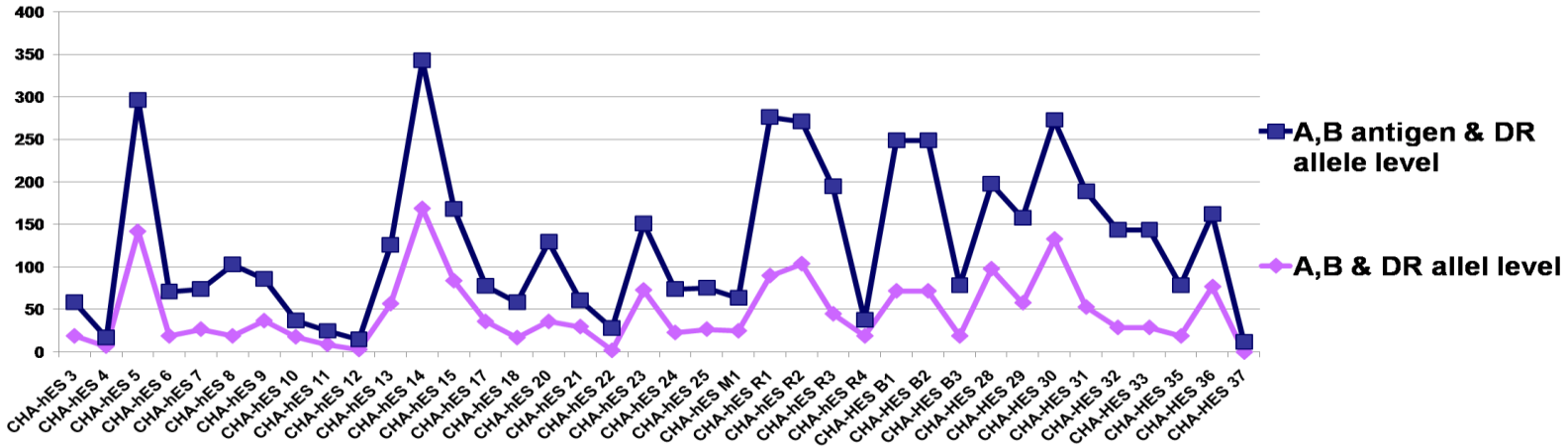
HLA and ABO genotypes of CHA-hESC lines (≥ 60)

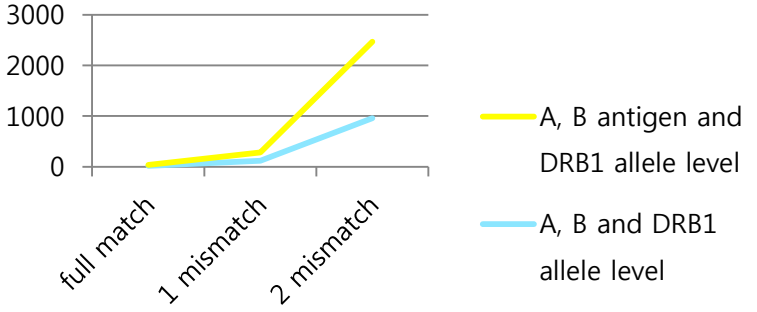
Cell Line	Result				ABO genotype
	HLA-Class I			HLA-Class II	
	HLA-A	HLA-B	HLA-Cw	HLA-DR	
CHA-hES 3	A* 2402, 3101	B* 1507, 4601	Cw* 0102, 0303[1]	DRB1* 0403, 0803	O/O
CHA-hES 4	A* 0207, 1101	B* 1801, 4601	Cw* 0102, 1203	DRB1* 0405, 1104	O/O
CHA-hES 5	A* 2402, 3303	B* 0702[1], 4403	Cw* 0702, 1403	DRB1* 0101[1], 0701	A/O
CHA-hES 6	A* 0201, 0203	B* 3802, 4001	Cw* 0304, 0702	DRB1* 0901, 1101	B/B
CHA-hES 7	A* 0201, 1101	B* 1511, 5401	Cw* 0102, 0303[1]	DRB1* 0405, 1202	O/O
CHA-hES 8	A* 1101, 2402	B* 1527, 4801	Cw* 0401[1], 0801[1]	DRB1* 0406, 1405	B/O
CHA-hES 9	A* 0201, 1101	B* 1301, 4001	Cw* 0304, 0702	DRB1* 1202, 1405	O/O
CHA-hES 10	A* 1101, 3004	B* 1401, 5401	Cw* 0102, 0802	DRB1* 0404, 1405	A/O
CHA-hES 11	A* 0201, 3101	B* 1518, 5401	Cw* 0102, 0704	DRB1* 0401, 1405	A/O
CHA-hES 12	A* 0203, 3004	B* 1401, 3802	Cw* 0702, 0802	DRB1* 0802, 1101	A/A
CHA-hES 13	A* 2402, 3001	B* 0702[1], 1302	Cw* 0602, 0702	DRB1* 0101[1], 0405	B/B
CHA-hES 14	A* 1101, 3303	B* 4403, 5101	Cw* 1402, 1403	DRB1* 0901, 1302	B/O
CHA-hES 15	A* 3101, 3303	B* 4403, 4601	Cw* 0102, 1403	DRB1* 0403, 1302	A/O
CHA-hES 16	N/A*	N/A*	N/A*	N/A*	O/O
CHA-hES 17	A* 3303, 6801	B* 5101, 5801	Cw* 0302, 1502	DRB1* 1302, 1407	A/O
CHA-hES 18	A* 2402, 2603	B* 1501, 5502	Cw* 0102, 0303	DRB1* 0901, 1501	B/O
CHA-hES 19	A* 0203, 2402	B* 3802, 5101	Cw* 0702, 1402	DRB1* 0803, 1502	B/O
CHA-hES 20	A* 0201, 0207	B* 1501, 4601	Cw* 0103, 0401[1]	DRB1* 0901, 1401[1]	B/O
CHA-hES 21	A* 0101, 3303	B* 3701, 4403	Cw* 0602, 0701	DRB1* 0301, 0701	A/O
CHA-hES 22	A* 0206, 0206	B* 4001, 5502	Cw* 0102, 0702	DRB1* 1202, 1501	O/O
CHA-hES 23	A* 2402, 3303	B* 4403, 4601	Cw* 0103, 1403	DRB1* 0901, 1501	A/O
CHA-hES 24	A* 0206, 3004	B* 1401, 3501[1]	Cw* 0801[1], 0802	DRB1* 0404, 1201[1]	A/B
CHA-hES 25	A* 0101, 0206	B* 3501[1], 3701	Cw* 0303[1], 0602	DRB1* 0405, 1001	O/O
CHA-hES 26	A* 0201, 0206	N/A*	Cw* 0102, 0304	DRB1* 0405, 1405	A/B
CHA-hES M1	A* 1101, 3101	B* 1501, 4002	Cw* 0304, 0401[1]	DRB1* 0406, 1407	A/O
CHA-hES R1	A* 0206, 2402	B* 0702[1], 5901	Cw* 0102, 0702	DRB1* 0101[1], 0405	O/O
CHA-hES R2	A* 0201, 3303	B* 2705[1], 5801	Cw* 0102, 0302	DRB1* 0101[1], 1302	B/O
CHA-hES R3	A* 0206, 0207	B* 4601, 5101	Cw* 0102, 1402	DRB1* 0803, 1201[1]	A/A
CHA-hES R4	A* 0301, 2402	B* 0702[1], 2705[1]	Cw* 0202, 0702	DRB1* 0101[1], 0101[1]	B/O

Embryonic Stem Cell Bank (HLA-matched Cell Bank)

Overcome of Immunological Problems after Transplantation - I

HLA typing of 38 CHA-hESC lines vs. 7387 donated cord bloods for simulation of stem cell transplantation





	Full match	1 mismatch	2 mismatch
A, B antigen and DRB1 allele level	0.31%	3.84%	41.49%
A, B and DRB1 allele level	0.24%	2.34%	24.34%

Lee et al., Cell Transplantation 2010, CHA Stem Cell Institute, 2011

It was estimated that our 38 CHA-hESC lines can provide a coverage for 27% and 45% of the Korean population with A, B, DR allele level and A, B antigen/DR allele level matches, respectively.

For the Clinical Application of Pluripotent Stem Cells

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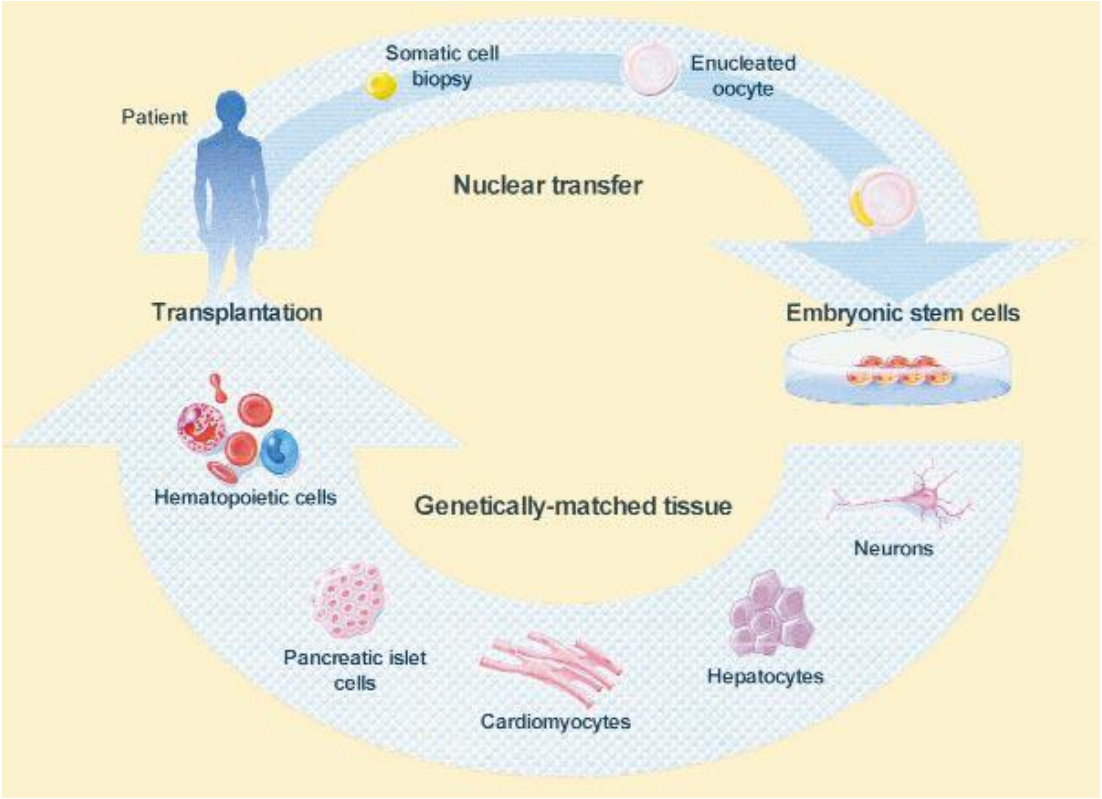
- Creation of universal cells by genetic modification to reduce the expression of MHC molecule
- **Establishment of large banks of immunophenotyped hESC lines** to match MHC alleles between hESC lines and patients

or

Generation of Isogenic Plutripotent Stem Cell Lines *using the patient's own somatic cells by* ***SCNT or IPS technologies***

Somatic Cell Nuclear Transferred (SCNT)- Stem Cells

Therapeutic cloning



- **produce pluripotent stem cells that carry the nuclear genome of the patient**
- **eliminate the critical problem of immune incompatibility**

Lanza et al., Nat Biotechnol 1999

Somatic Cell Nuclear Transferred (SCNT)- Stem Cells

Difficulties in derivation of SCNT-stem cells

ARTICLE

doi:10.1038/nature10397

Human oocytes reprogram somatic cells to a pluripotent state

Scott Noggle[1], Ho-Lim Fung[2], Athurva Gore[2], Hector Martinez[1], Kathleen Crumm Satriani[3,4], Robert Prosser[3,4], Kiboong Oum[3,4], Daniel Paull[1], Sarah Druckenmiller[1], Matthew Freeby[5,6], Ellen Greenberg[5,6], Kun Zhang[2], Robin Goland[5,6], Mark V. Sauer[3,4], Rudolph L. Leibel[5,6] & Dieter Egli[1]

Noggle et al., Nature 2011

STEM CELLS

TECHNOLOGY DEVELOPMENT

Development of Human Cloned Blastocysts Following Somatic Cell Nuclear Transfer with Adult Fibroblasts

ANDREW J. FRENCH,[a] CATHARINE A. ADAMS,[b] LINDA S. ANDERSON,[b] JOHN R. KITCHEN,[c] MARCUS R. HUGHES,[c] SAMUEL H. WOOD[a,b]

French et al., Stem Cells 2008

Stem Cells Dev. 2011 Nov;20(11):1951-9. doi: 10.1089/scd.2010.0451. Epub 2011 Apr 13.

Derivation of cloned human blastocysts by histone deacetylase inhibitor treatment after somatic cell nuclear transfer with β-thalassemia fibroblasts.

Fan Y[1], Jiang Y, Chen X, Ou Z, Yin Y, Huang S, Kou Z, Li Q, Long X, Liu J, Luo Y, Liao B, Gao S, Sun X.

Fan et al., Stem Cells Dev 2011

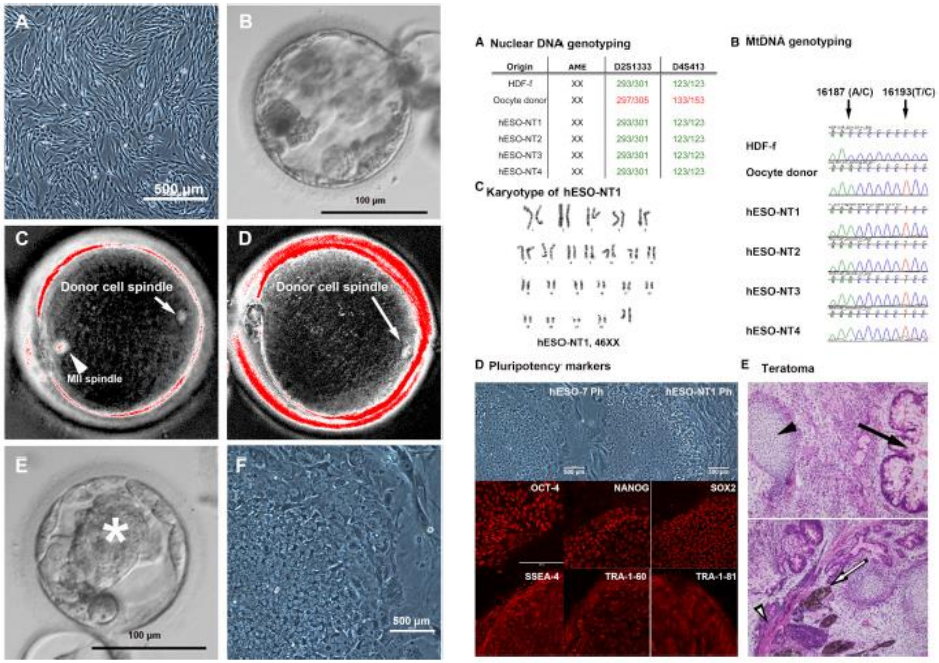
- **SCNT embryos fail to progress beyond the 8-cell stage or did reach the blastocyst stage only.**
- **may due to an inability to reprogram critical embryonic genes from the somatic donor cell nucleus**

Somatic Cell Nuclear Transferred-Stem Cells

First successful derivation of SCNT-SCs using fetal and neonatal somatic donor cells

Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

Masahito Tachibana,[1] Paula Amato,[2] Michelle Sparman,[1] Nuria Marti Gutierrez,[1] Rebecca Tippner-Hedges,[1] Hong Ma,[1] Eunju Kang,[1] Alimujiang Fulati,[1] Hyo-Sang Lee,[1,6] Hathaitip Sritanaudomchai,[3] Keith Masterson,[2] Janine Larson,[2] Deborah Eaton,[2] Karen Sadler-Fredd,[2] David Battaglia,[2] David Lee,[2] Diana Wu,[2] Jeffrey Jensen,[1,4] Phillip Patton,[2] Sumita Gokhale,[5] Richard L. Stouffer,[1,2] Don Wolf,[1] and Shoukhrat Mitalipov[1,2,*]

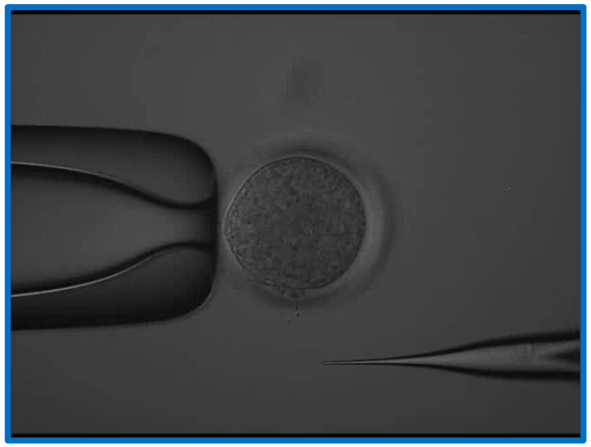


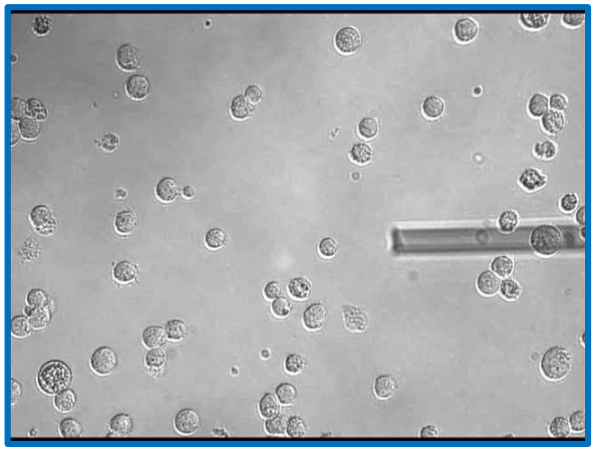
Tachibana et al., Cell 2013

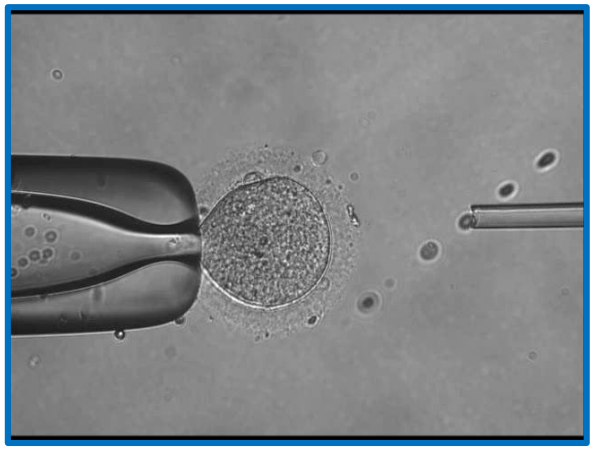
- The use of electrical activation combined with ionomycine /DMAP activation
- The use of HDAC inhibitor after oocyte activation
- **Caffeine** treatment during enucleation
- The use of a hormone stimulation protocol yielding a small number of **high quality oocytes**

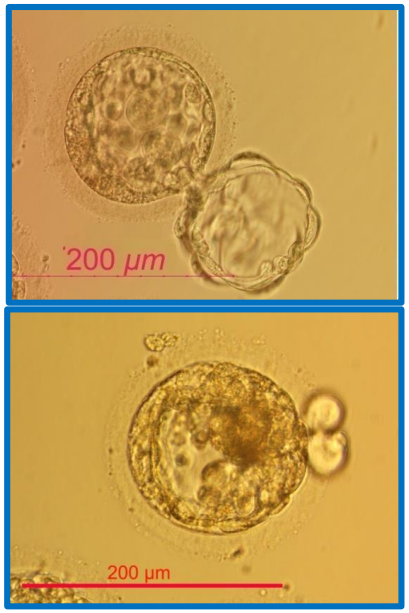
- Derivation of 4 SCNT-SC lines using same fetal embryonic fibroblasts
- Derivation of 2 SCNT-SC lines using cells from an 8-month-old subject with Leigh syndrome (but, no karyotype or evidence of pluripotency was provided)

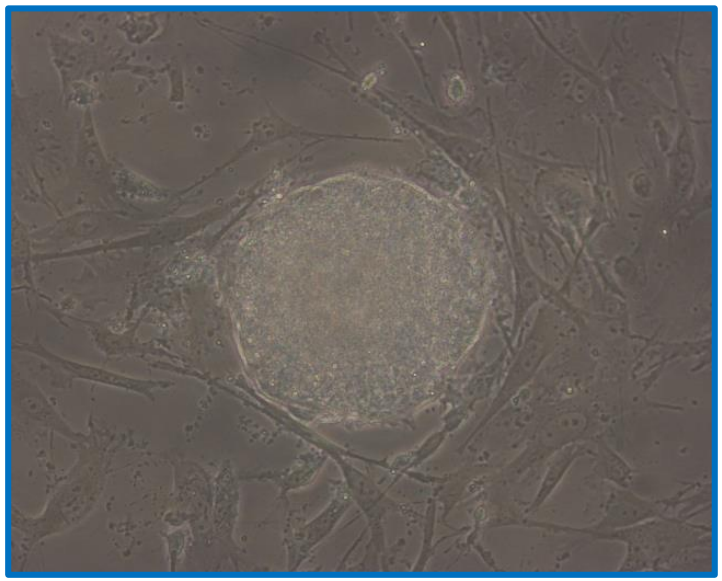
SCNT– derived stem cells (SCNT-SCs)











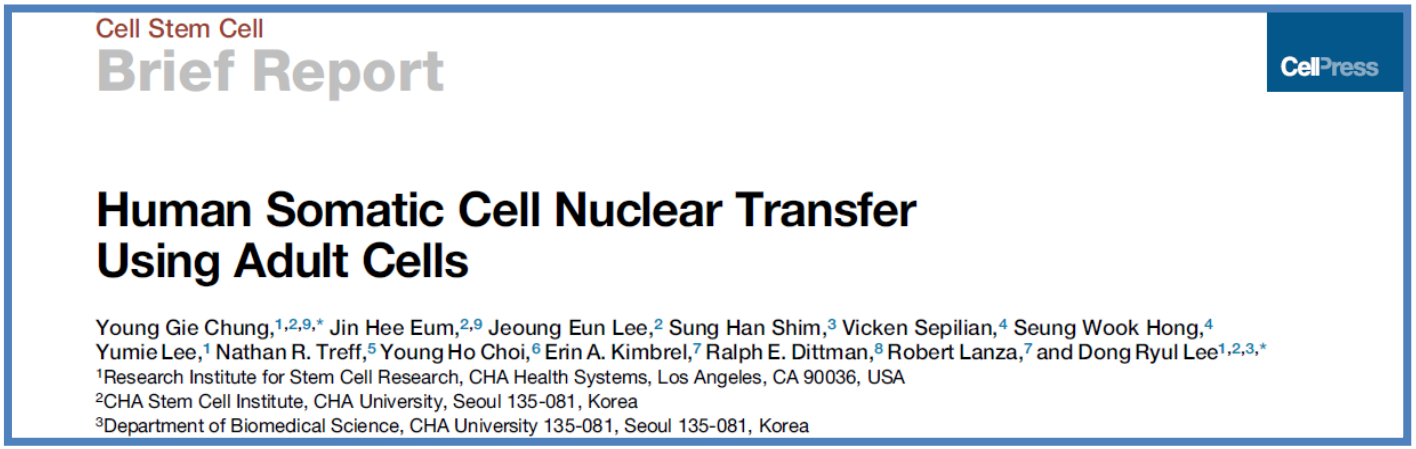
CHA NT2

Sources of patient-specific pluripotent stem cells

- patient-specific pluripotent stem cells

  - induced pluripotent stem cells (iPSCs)

  - **Somatic cell nuclear transfer –derived stem cells (SCNT-SCs)**



Cell Stem Cell

Brief Report

CellPress

Human Somatic Cell Nuclear Transfer Using Adult Cells

Young Gie Chung,[1,2,9,*] Jin Hee Eum,[2,9] Jeoung Eun Lee,[2] Sung Han Shim,[3] Vicken Sepilian,[4] Seung Wook Hong,[4] Yumie Lee,[1] Nathan R. Treff,[5] Young Ho Choi,[6] Erin A. Kimbrel,[7] Ralph E. Dittman,[8] Robert Lanza,[7] and Dong Ryul Lee[1,2,3,*]

[1]Research Institute for Stem Cell Research, CHA Health Systems, Los Angeles, CA 90036, USA

[2]CHA Stem Cell Institute, CHA University, Seoul 135-081, Korea

[3]Department of Biomedical Science, CHA University 135-081, Seoul 135-081, Korea

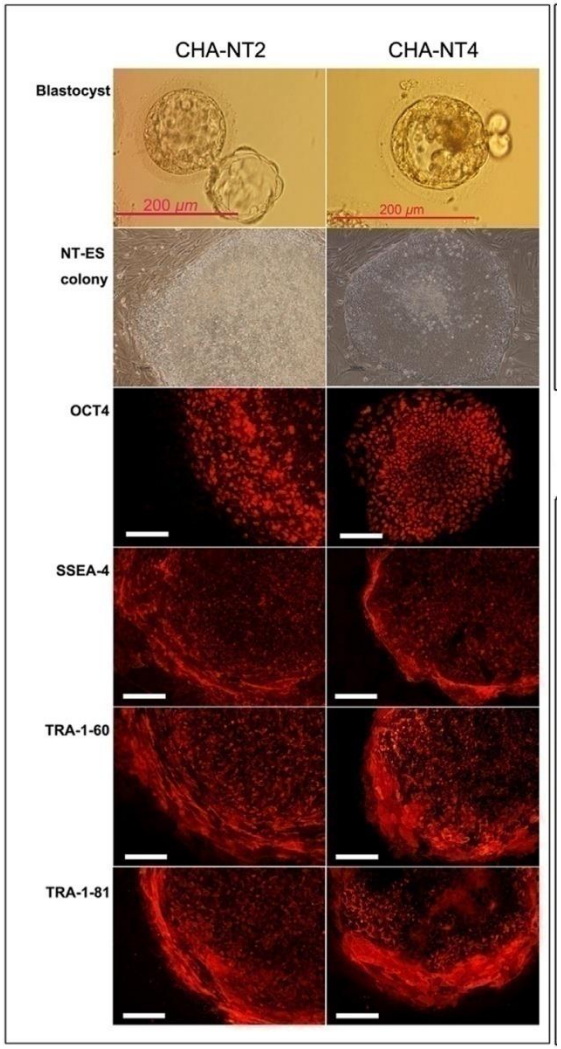
- ***Derivation of human SCNT-stem cells using adult somatic cells has potential for application in a range of therapeutic contexts.***

- ***2 human SCNT-SC lines using dermal fibroblasts from 35- and 75-year-old males.***

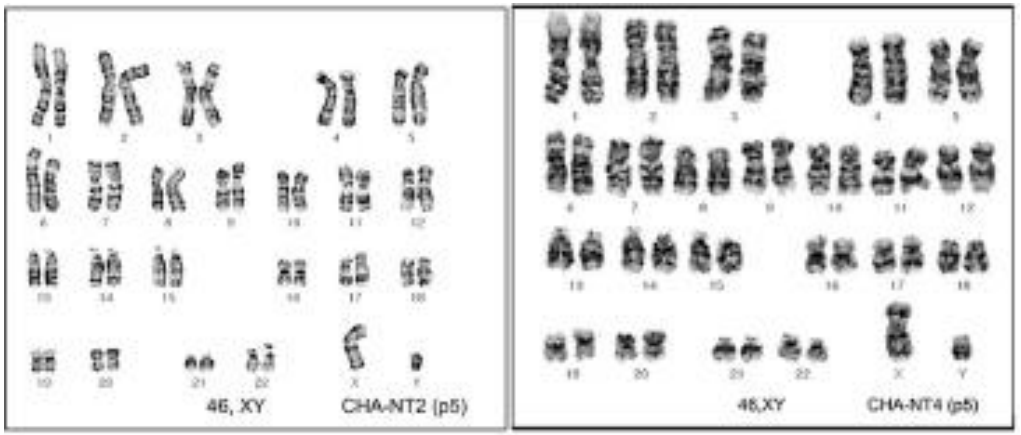
Chung et al., Cell Stem Cells 2014

Establishment of SCNT-Stem Cells using Adult Cells

- **Derivation of SCNT-SC lines and Characterization**



Karyotypes of SCNT-SC lines



Chung et al., Cell Stem Cells 2014

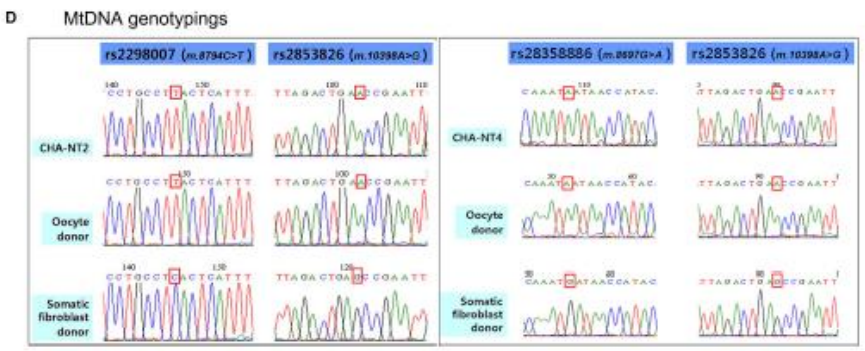
Establishment of SCNT-Stem Cells using Adult Cells

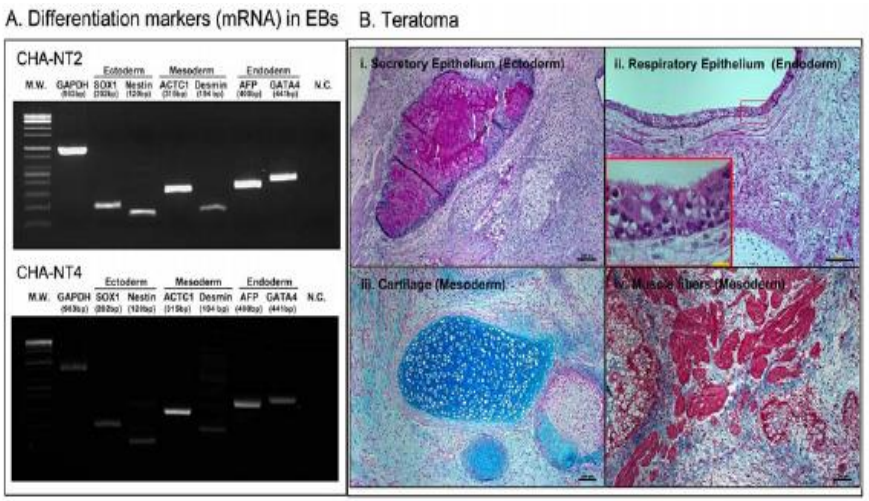
- **Characterization of SCNT-SC lines and Characterization**

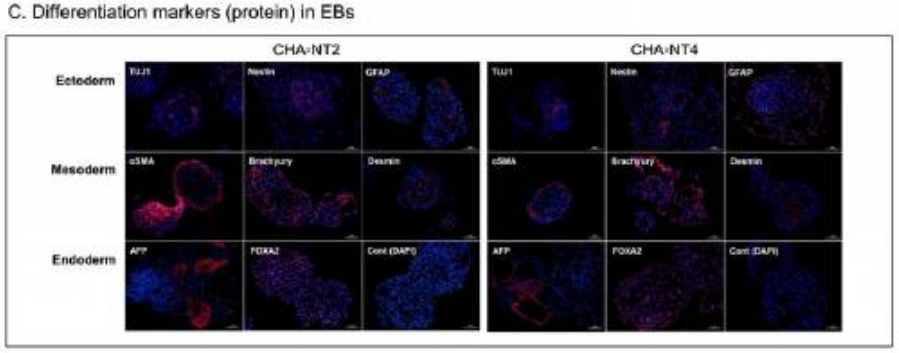
Nuclear DNA genotyping

	Fibroblast (DFB-2)		CHA-NT2		Oocyte donor	
D8S1179	14	16	14	16	13	15
D21S11	28	30	28	30	29	31.2
D7S820	9	10	9	10	11	11
CSF1PO	12	12	12	12	10	12
D3S1358	15	17	15	17	16	16
TH01	6	7	6	7	6	6
D13S317	9	11	9	11	9	12
D16S539	11	12	11	12	10	11
D2S1338	17	19	17	19	17	20
D19S433	14	14	14	14	13.2	16
vWA	17	19	17	19	14	19
TPOX	8	10	8	10	11	12
D18S51	14	19	14	19	12	17
AMEL	X	Y	X	Y	X	X
D5S818	11	12	11	12	11	12
FGA	22	23	22	23	23	26

	Fibroblast (DFB-1)		CHA-NT4		Oocyte donor	
D8S1179	14	14	14	14	13	15
D21S11	28	29	28	28	29	30
D7S820	11	12	11	12	8	11
CSF1PO	10	12	10	12	10	12
D3S1358	14	17	14	17	16	17
TH01	6	9	6	9	9	9.3
D13S317	8	9	8	9	11	11
D16S539	11	12	11	12	12	12
D2S1338	18	22	18	22	21	25
D19S433	15.2	16.2	15.2	16.2	13	13
vWA	16	17	16	17	16	17
TPOX	8	11	8	11	8	8
D18S51	14	16	14	16	12	13
AMEL	X	Y	X	Y	X	X
D5S818	11	12	11	12	11	12
FGA	19	23	19	23	19	21



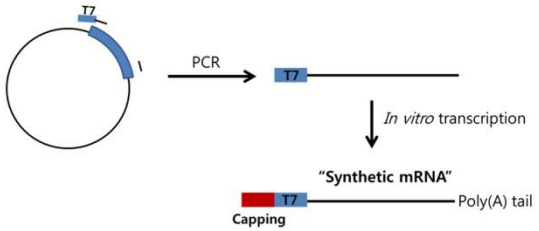




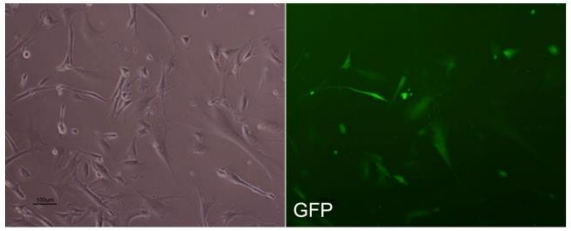
Chung et al., Cell Stem Cells 2014

Derivation-efficiency of human SCNT-SC lines

A. Schematic procedures for generating synthetic mRNAs



B. Expression of synthetic mRNAs in human fibroblat cells



C. Expression of syntheic mRNAs in human enucleated oocytes

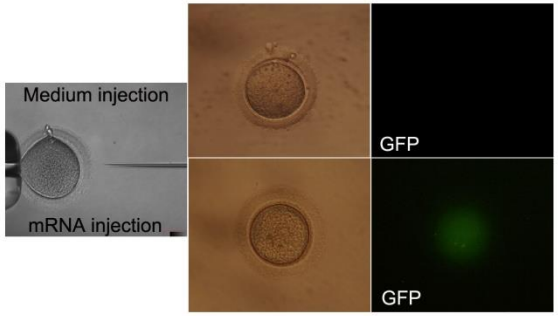


Table 1. SCNT Human Embryo Development from Two Nuclear Donors

			Embryo Development							
Treatment	Nuclear Donor[b]	Oocyte Donor	Total	Two-Cell	Four-Cell	Eight-Cell	Morula	Blastocysts	Hatching Blastocysts	ESC Line
30 min	DFB-2	DOE-D	12	11	11	8	2	1	0	0
		DOE-E	12	11	8	8	3	0	0	0
	DFB-1	DOE-F	9	8	7	6	2	0	0	0
		DOE-G	5	3	3	3	2	1	0	0
	Subtotal		38	33	29	25	9	2	0	0
2 hr	DFB-2	DOE-D	12	11	10	9	4	2	1	1[a]
		DOE-E	12	11	9	9	3	0	0	0
	DFB-1	DOE-F	10	8	8	6	2	0	0	0
		DOE-G	5	4	4	4	3	1	1	1[a]
	Subtotal		39	34	31	28	12	3	2	2[a]

DFB-1: 35-year-old male; DFB-2: 75-year-old male. Incubation time prior to activation (30 min versus 2 hr) does not drastically alter cloning efficiency; however, the hatching blastocysts were obtained only from the 2 hr group. See also Table S1 and Table S2.

[a]Karyotyping showed normal 46 XY diploidy.

[b]There were no significant differences between the two treatment groups at any developmental stage (X^2-test, $p > 0.05$).

1. Injection with mRNA for two centrosomal proteins (Human NuMA and HSET) into reconstituted eggs.

   ➔ did not enhance cloning efficiency

2. The slightly prolonged incubation time (30 min to 2 hours) before activation

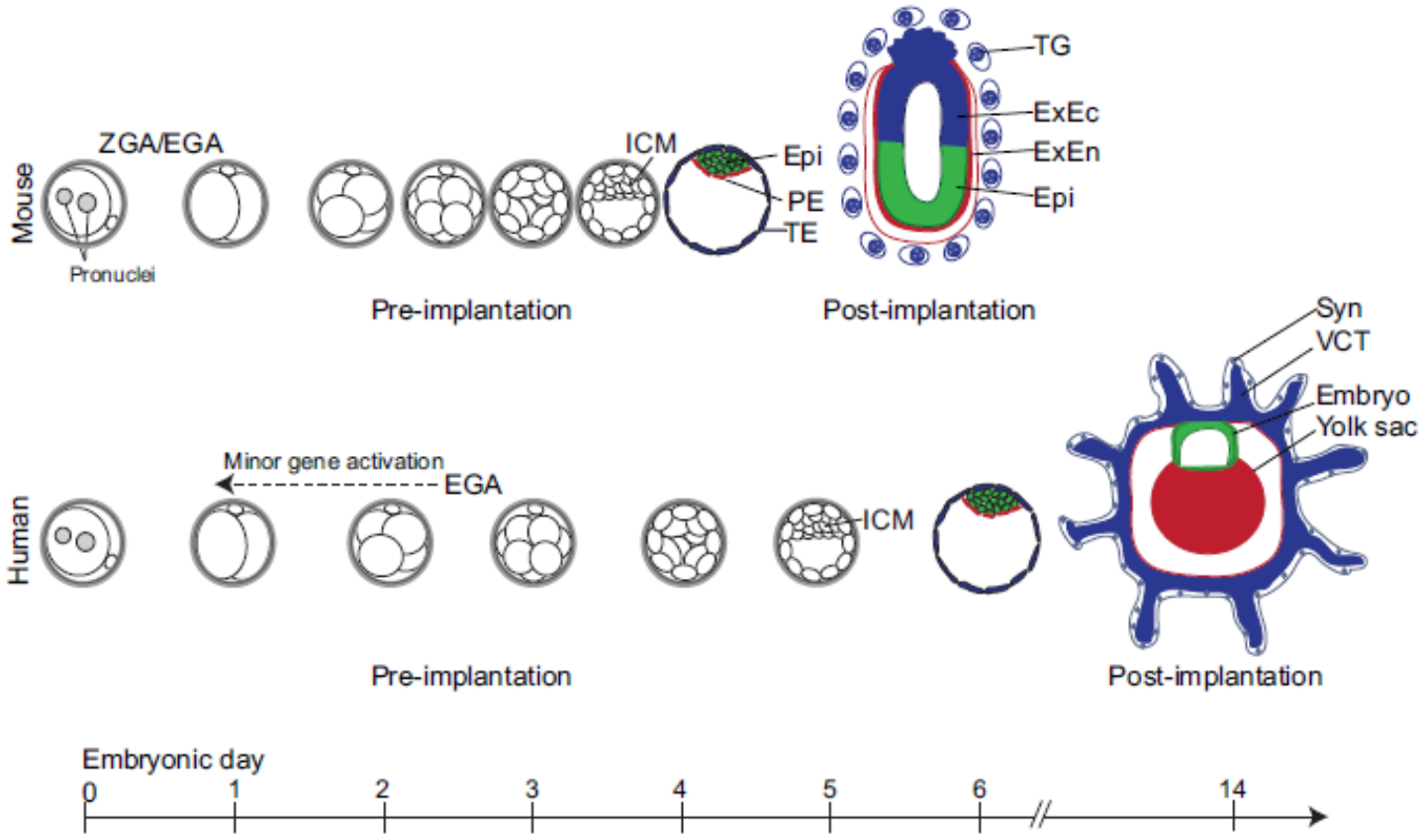
   ➔ may enhance developmental progression of cloned embryos.

3. SCNT-SC lines were derived from only hatching blastocysts at day 6 and specific oocyte donors.

Chung et al., Cell Stem Cells 2014

Still extremely low efficiency !!!

Cell fate decisions and their timing in mouse versus human early embryo development.



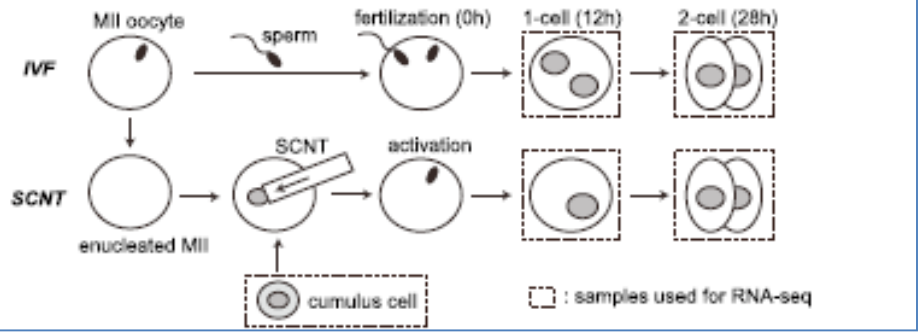
Niakan et al., Development 2012

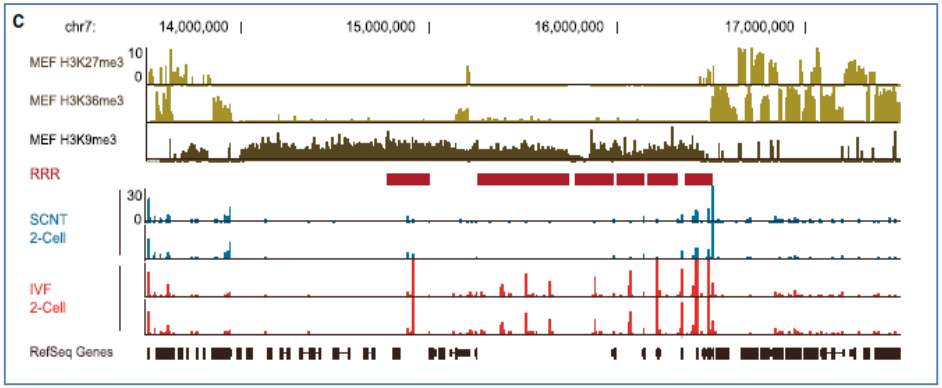
Cell

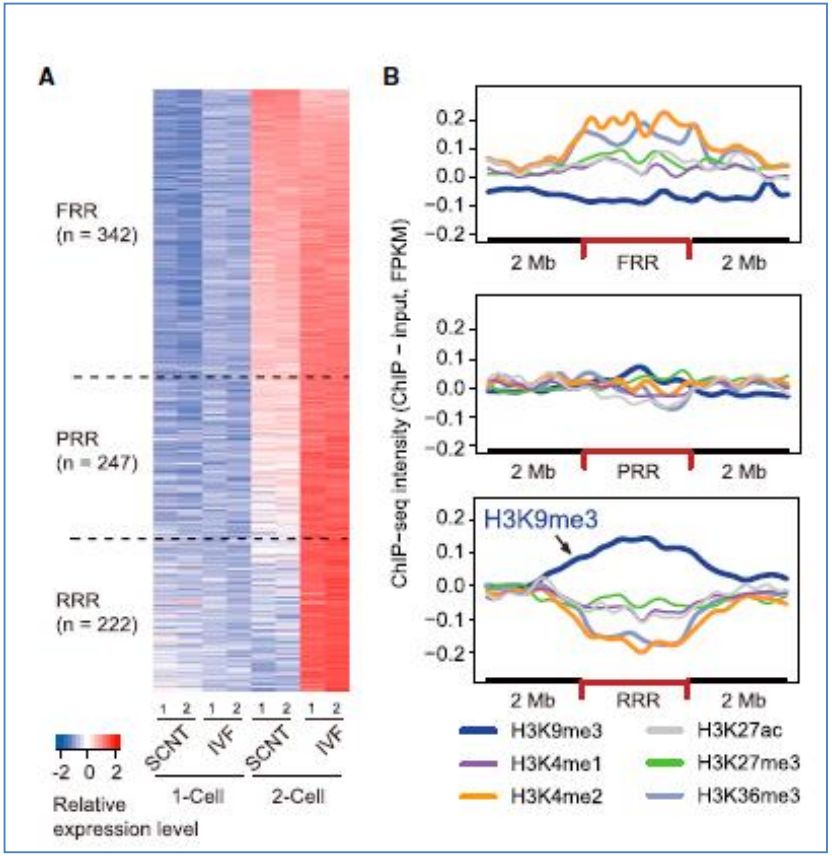
Article

Embryonic Development following Somatic Cell Nuclear Transfer Impeded by Persisting Histone Methylation

Shogo Matoba,[1,2,3,5] Yuting Liu,[1,2,3,5] Falong Lu,[1,2,3] Kumiko A. Iwabuchi,[1,2,3] Li Shen,[1,2,3] Azusa Inoue,[1,2,3] and Yi Zhang[1,2,3,4,*]







Matoba et al., Cell 2014

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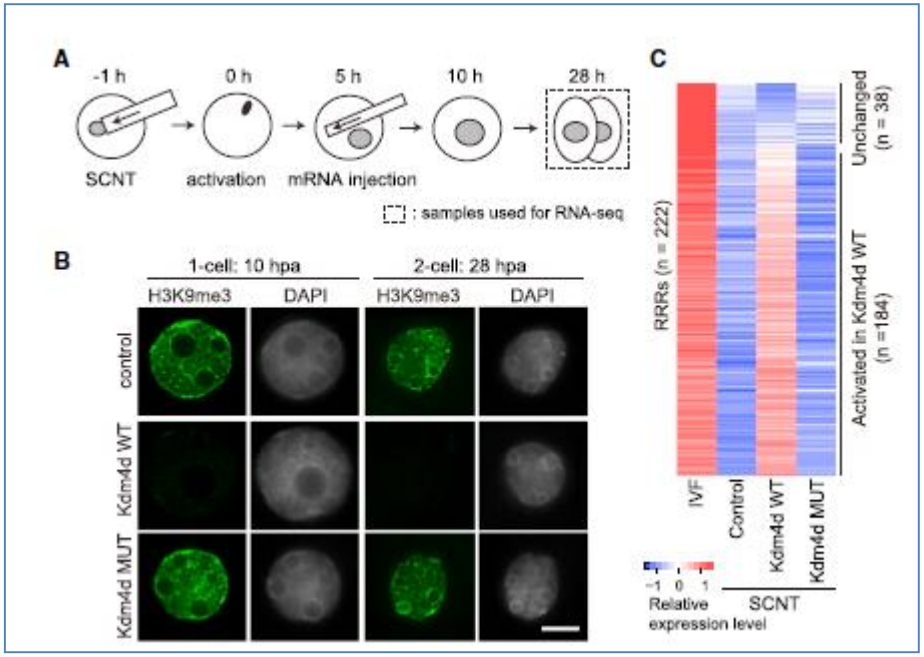
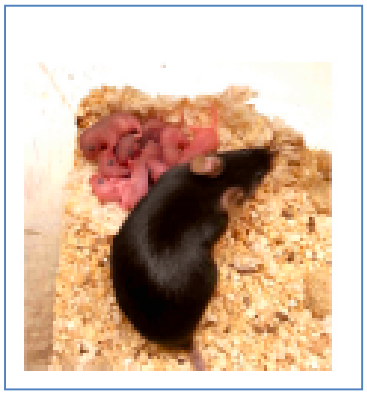
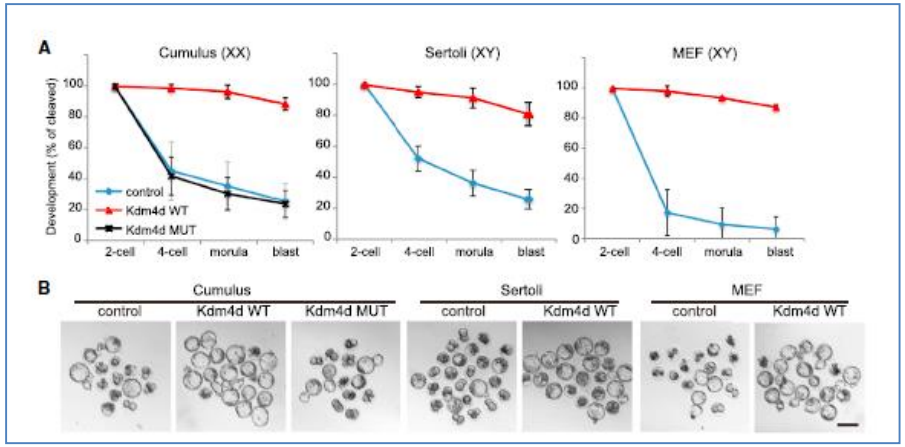
Shogo Matoba,[1,2,3,5] Yuting Liu,[1,2,3,5] Falong Lu,[1,2,3] Kumiko A. Iwabuchi,[1,2,3] Li Shen,[1,2,3] Azusa Inoue,[1,2,3] and Yi Zhang[1,2,3,4,*]

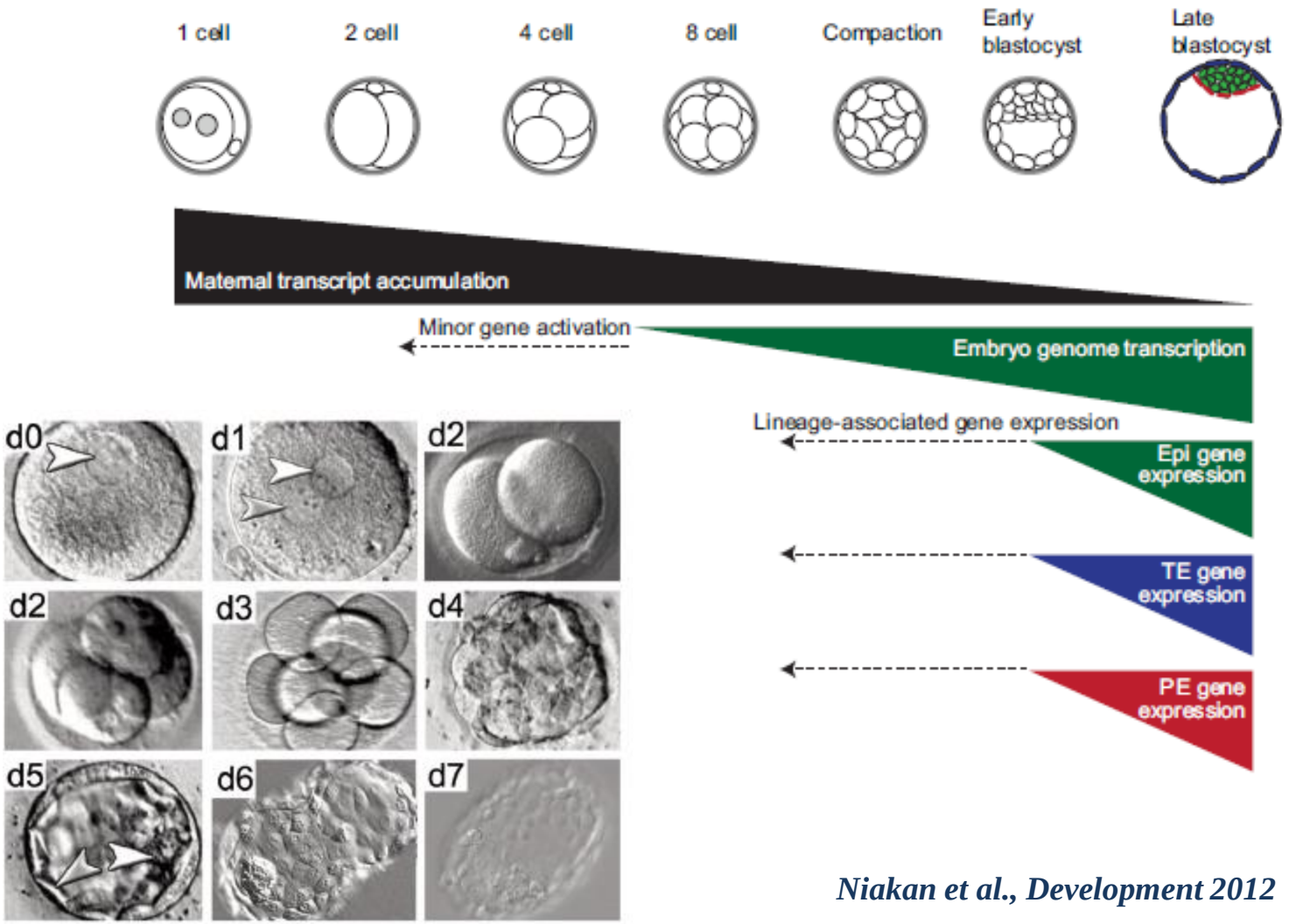






Matoba et al., Cell 2014

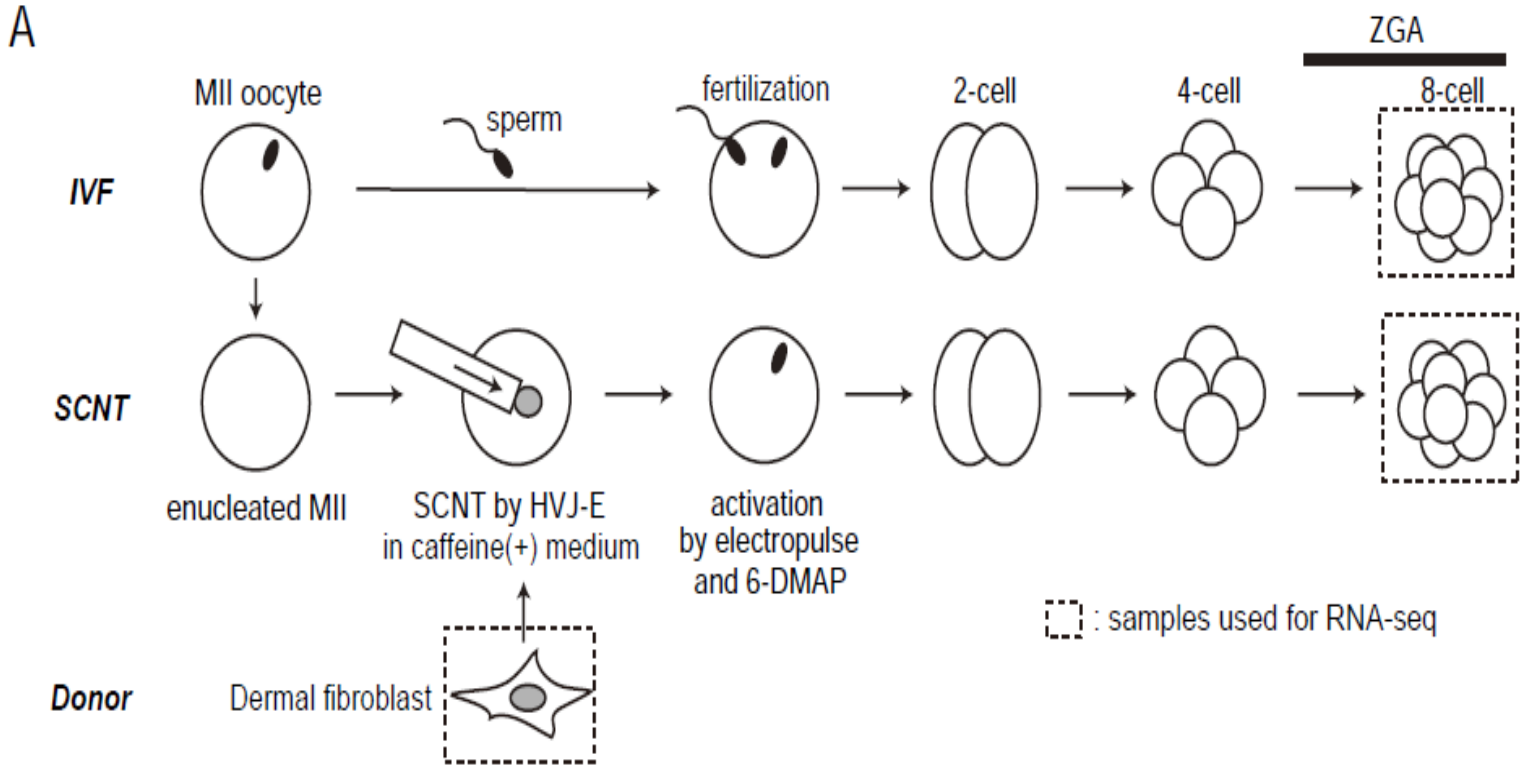
Genetic networks of human pre-implantation development



Niakan et al., Development 2012

Establishment of SCNT-Stem Cells using Adult Cells

- **Establishment-Efficiency of SCNT-SC lines using mRNAs of reprogramming-factors**



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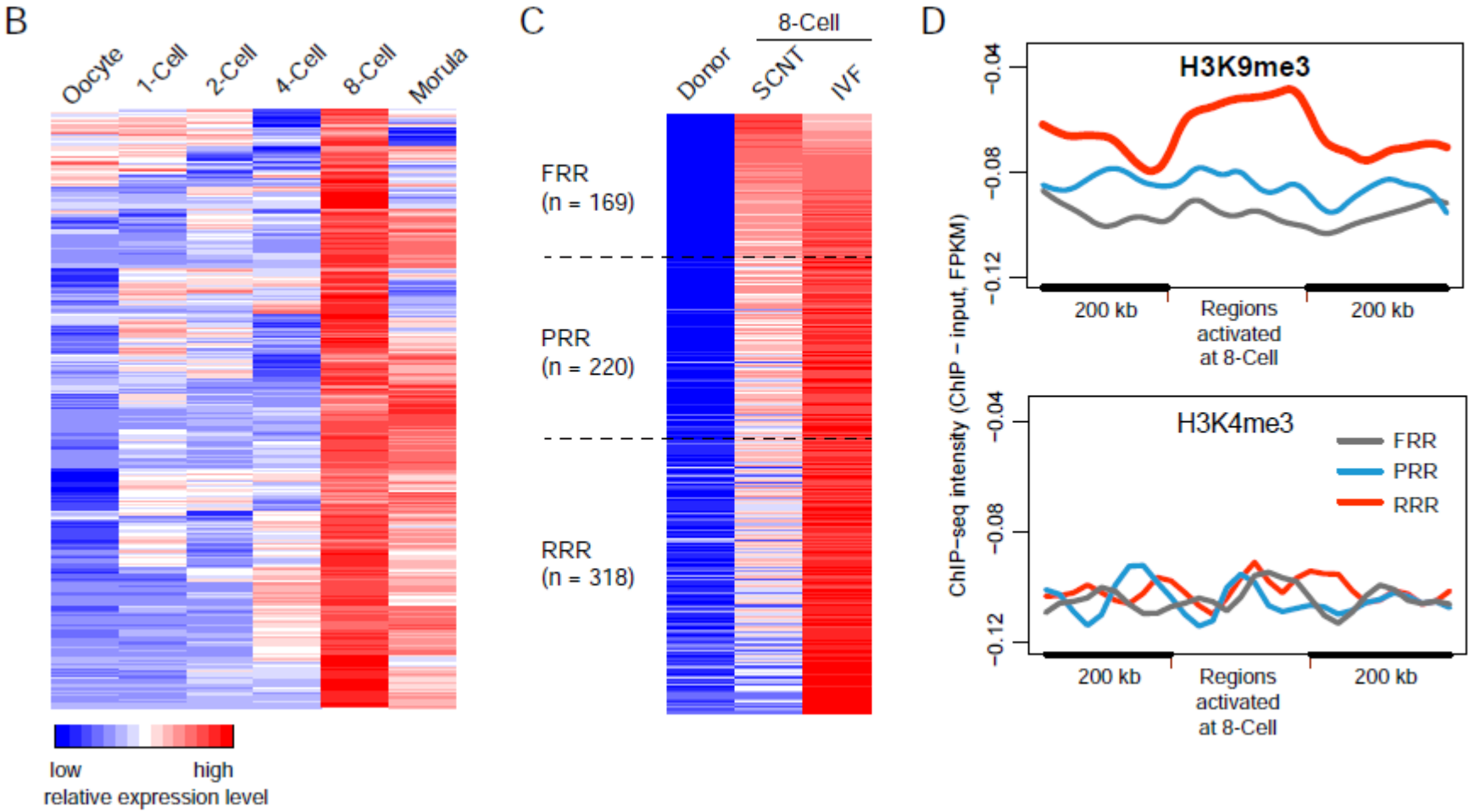
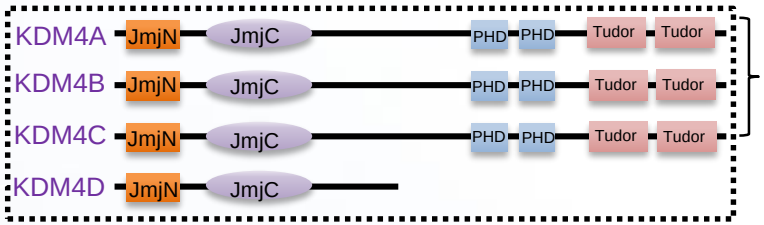
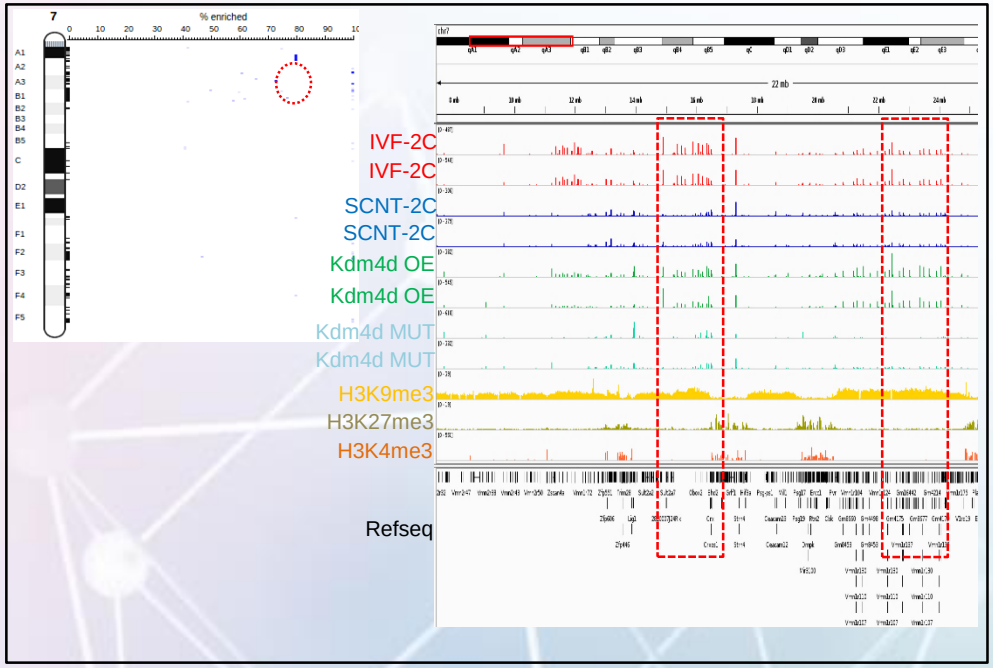


Kdm4d-dependent and independent modules

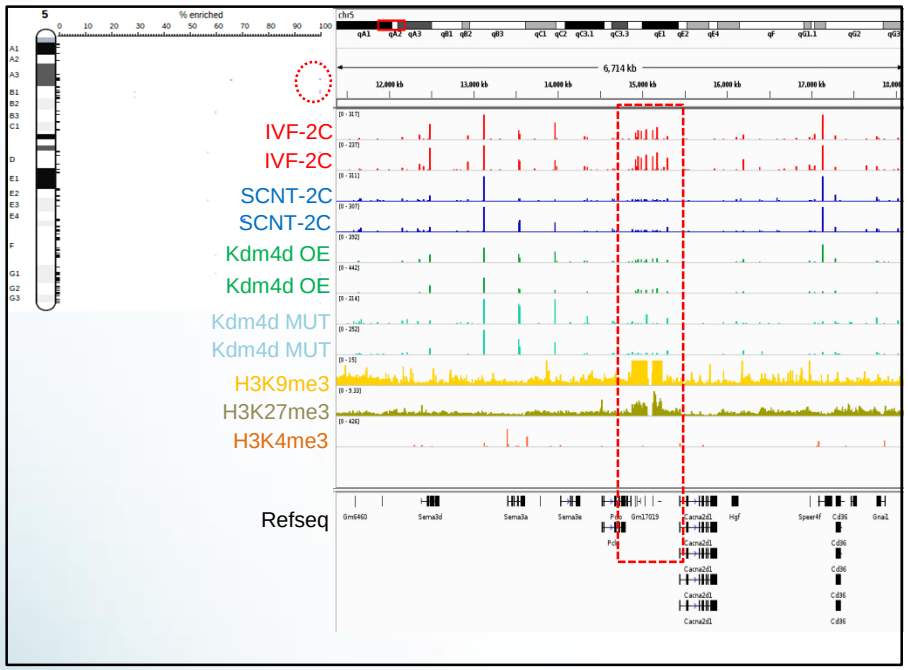


?

(Kdm4d-dependent)



(Kdm4d-independent)



(data from Cell 159: 884–895, 2014)

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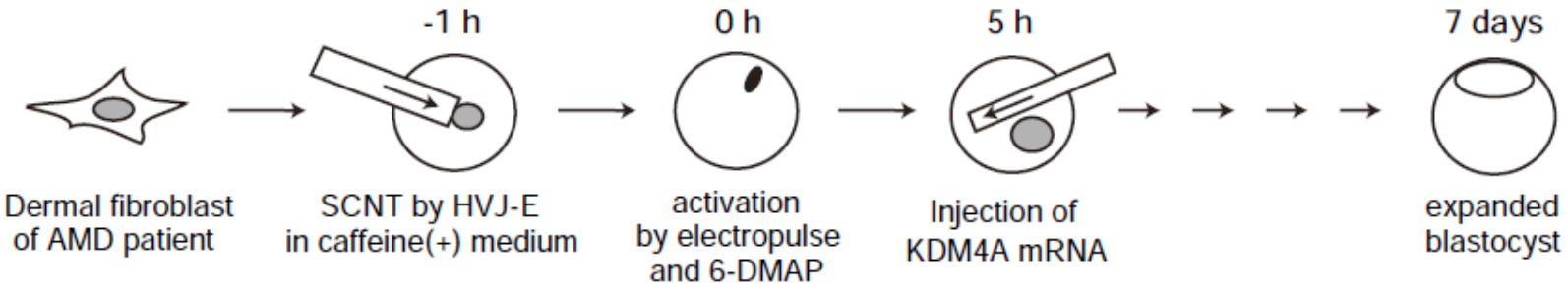

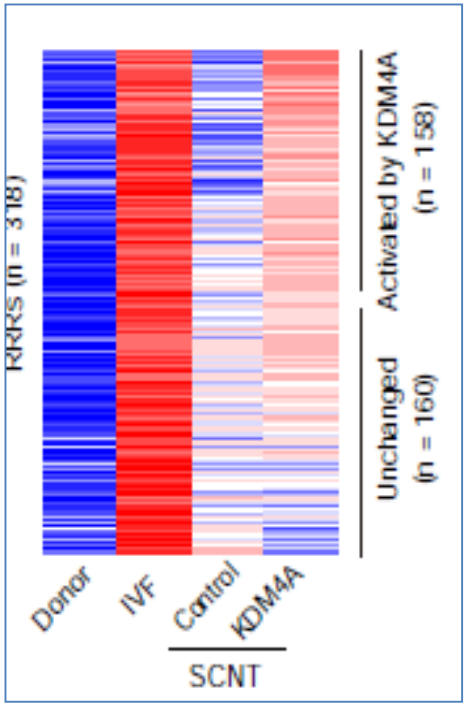

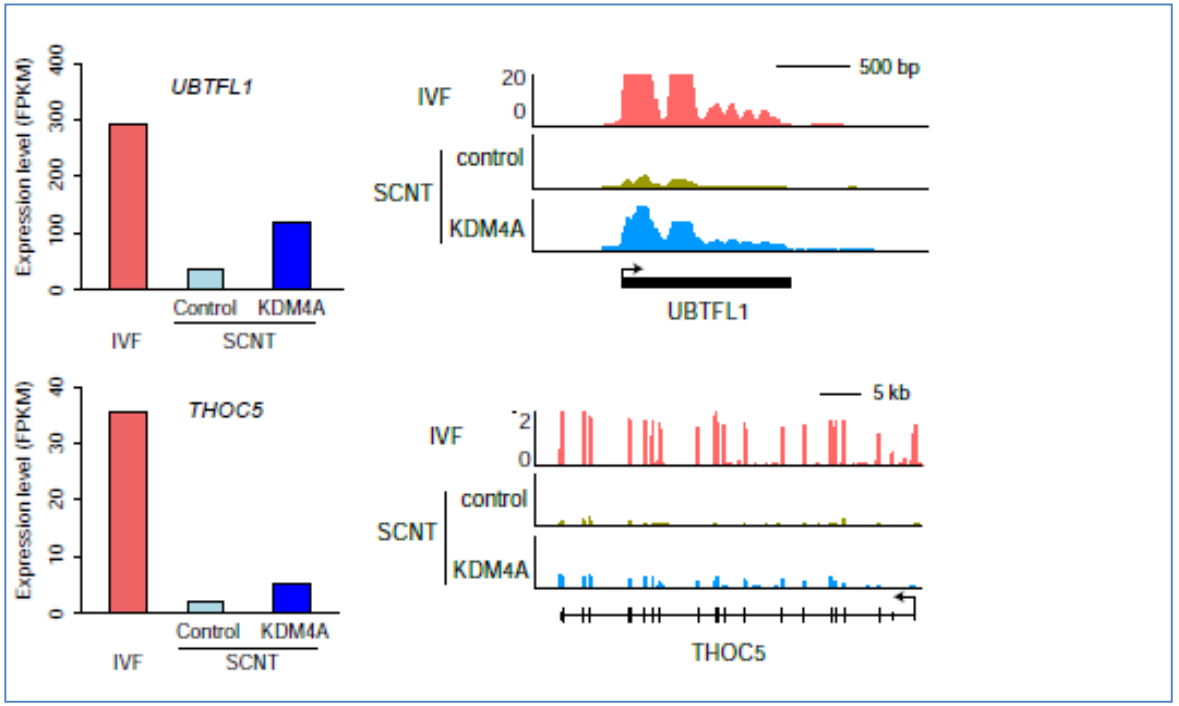

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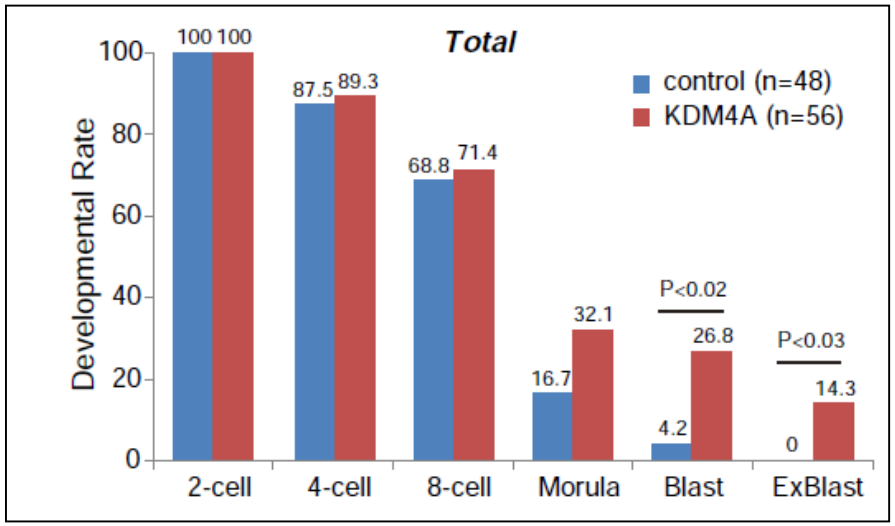

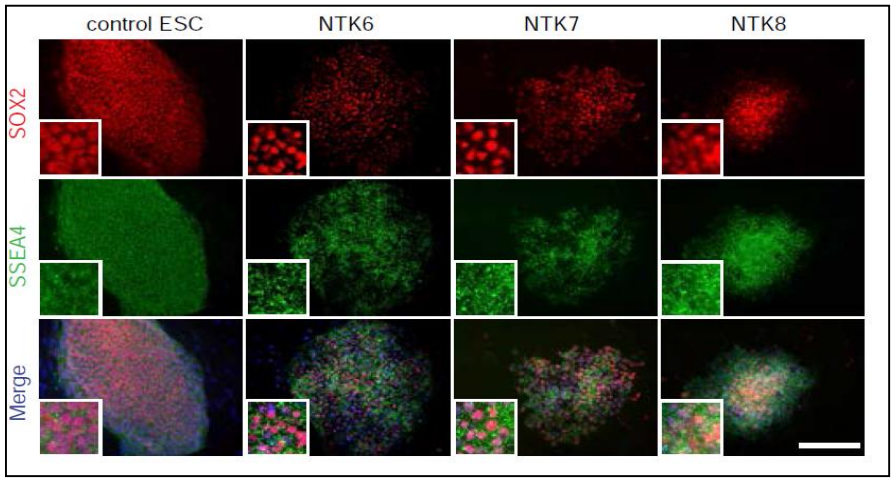

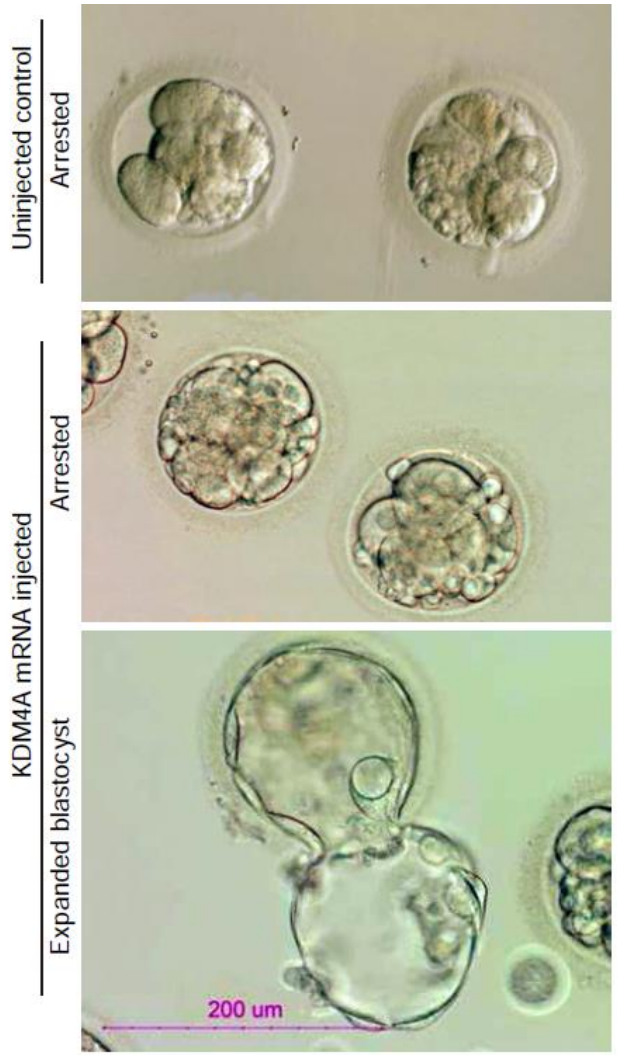

Chung et al., 2015 submitted

Establishment of SCNT-Stem Cells using Adult Cells

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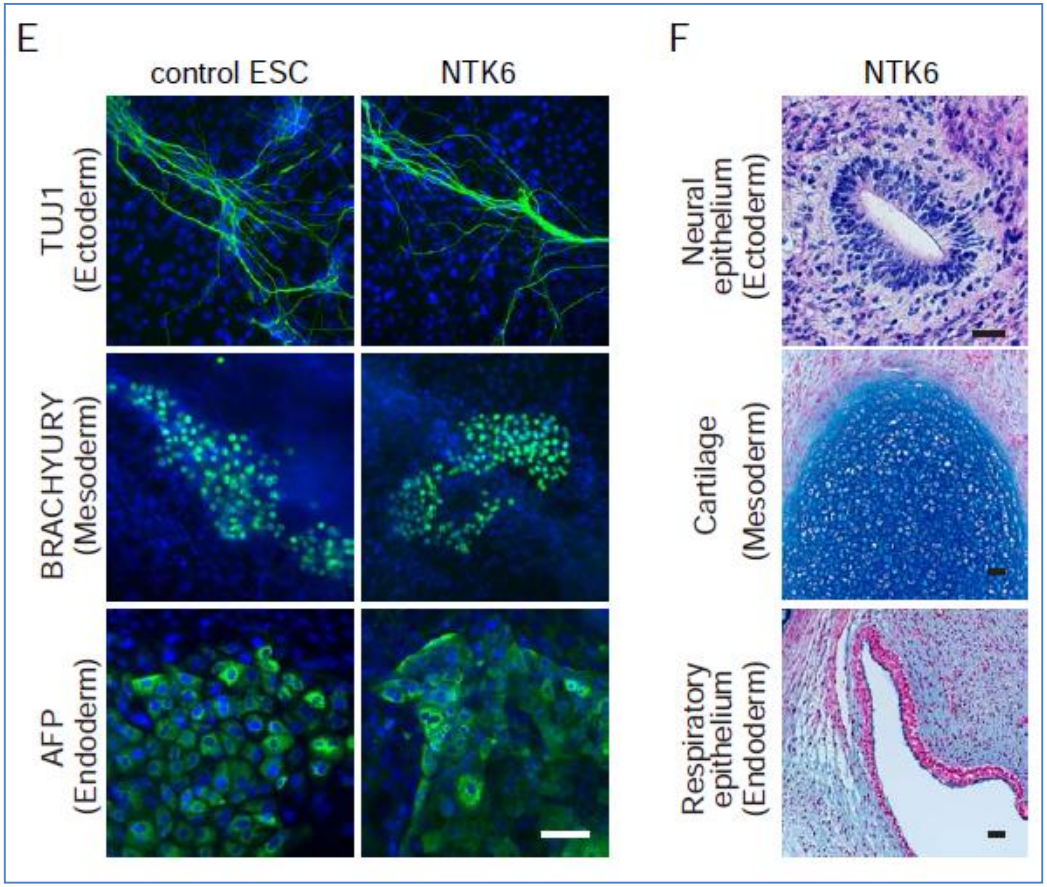
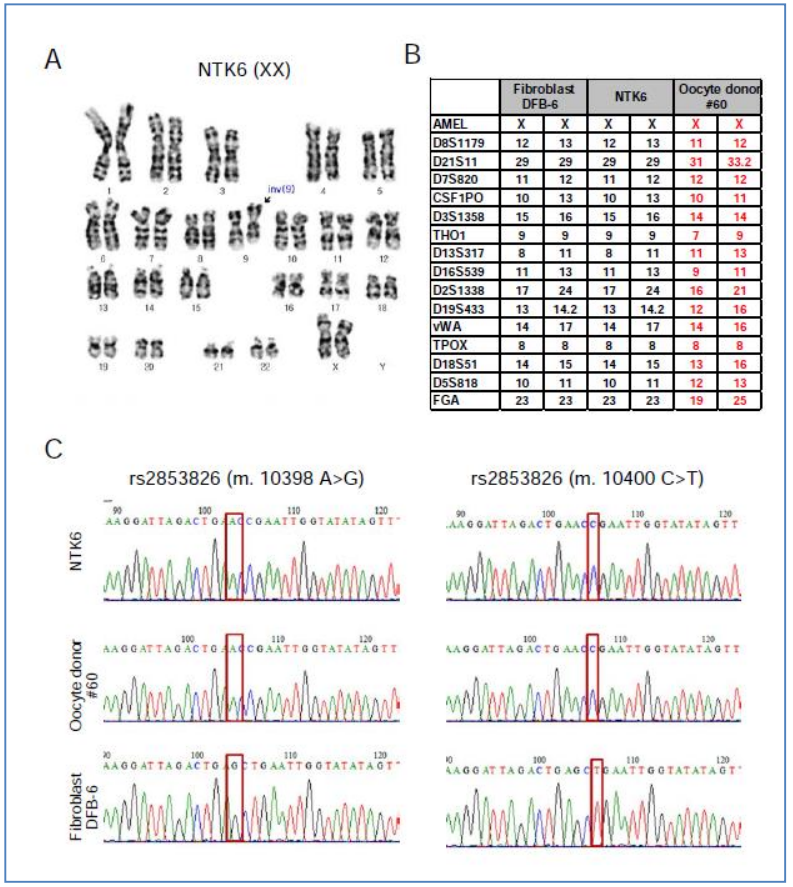
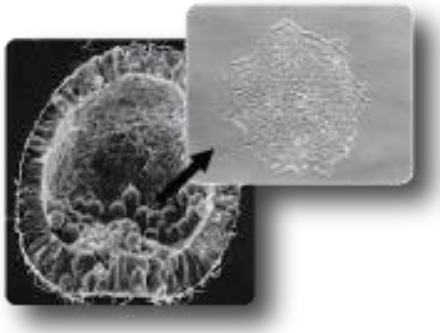




	Fibroblast DFB-6		NTK6		Oocyte donor #60	
AMEL	X	X	X	X	X	X
D8S1179	12	13	12	13	11	12
D21S11	29	29	29	29	31	33.2
D7S820	11	12	11	12	12	12
CSF1PO	10	13	10	13	10	11
D3S1358	15	16	15	16	14	14
THO1	9	9	9	9	7	9
D13S317	8	11	8	11	11	13
D16S539	11	13	11	13	9	11
D2S1338	17	24	17	24	16	21
D19S433	13	14.2	13	14.2	12	16
vWA	14	17	14	17	14	16
TPOX	8	8	8	8	8	8
D18S51	14	15	14	15	13	16
D5S818	10	11	10	11	12	13
FGA	23	23	23	23	19	25

Chung et al., 2015 submitted

Ideal Cell Sources for Stem Cell Therapy???



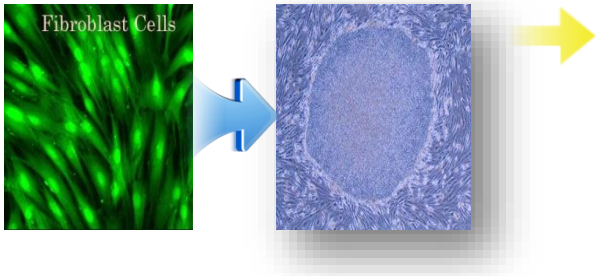
Embryonic Stem Cells (ESCs)

Fertilized embryo-derived ESCs

Parthenogenic embryo-derived ESCs

Single blastomere-derived ESCs

Mid brain tissue from aborted fetus

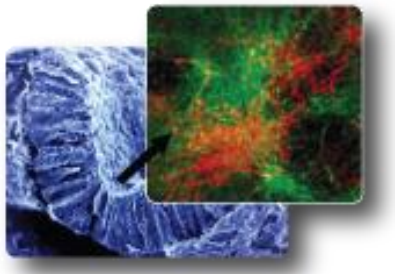


Reprogrammed Pluripotent Stem Cells

Somatic Cell Nuclear Transferred (SCNT)- Stem Cells

Induced Pluripotent Stem Cells (iPSCs)

Ideal Cells for Application



Autologous Adult Stem Cells

Adipose-derived

Peripheral blood-derived

Bone marrow-derived

Testicular stem cells (HTSC)

Allogeneic Adult Stem Cells

Cord blood-derived

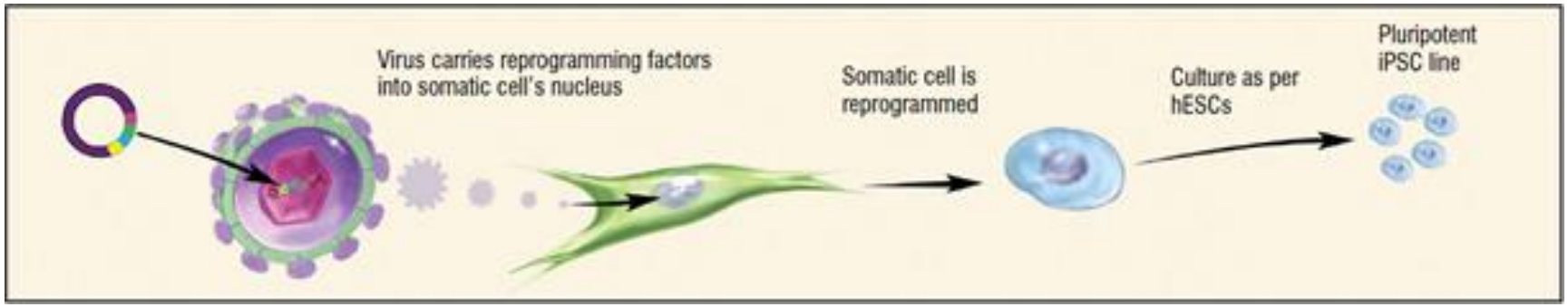
Placenta-derived

Wharton's jelly

Induced Pluripotent Stem Cells (IPSC)

Overcome of Immune recognition after Transplantation

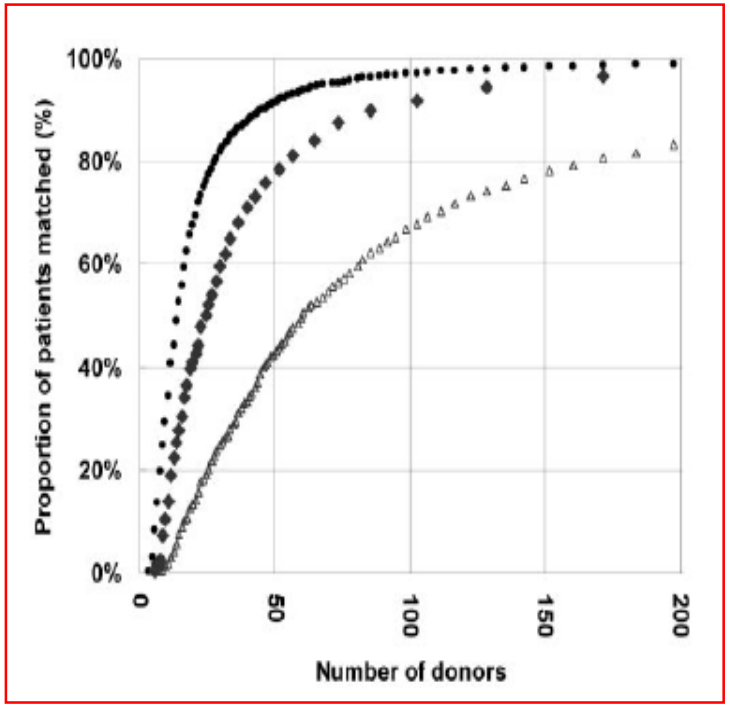
- Using 4 factors: Oct4, Sox2, Nanog, & Klf4, ES like cell production from patients' somatic cells (Takahashi and Yamanaka, 2006; Takahashi et al., 2007)
- However, clinical application of iPSC remains the safety issue with use of virus and gene modification



Goldthwaite Reg Med 2011

How many homozygous-NT-hESC lines are needed for clinical use?

Nakajima et al., Stem Cells 2006



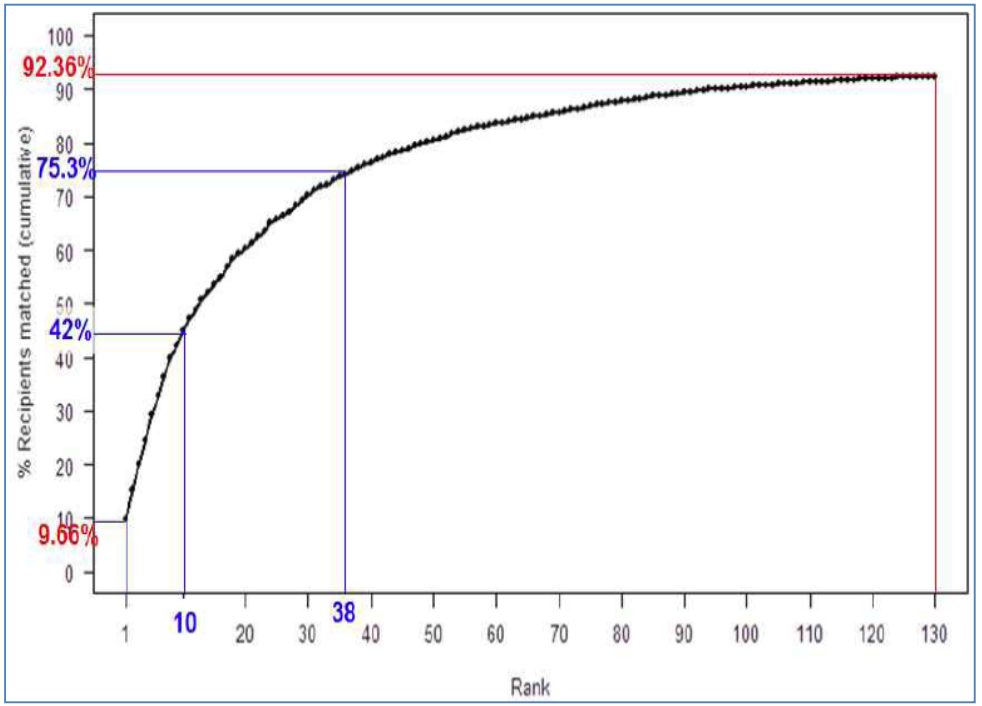
Cummulative proportion of patients with at least one human HLA-matched Parthenogenetic donor for hESC transplantation

◆: Parthenogenetic donor with full match;

△: single mismatch at one loci or HLA-A, -B, or –DR

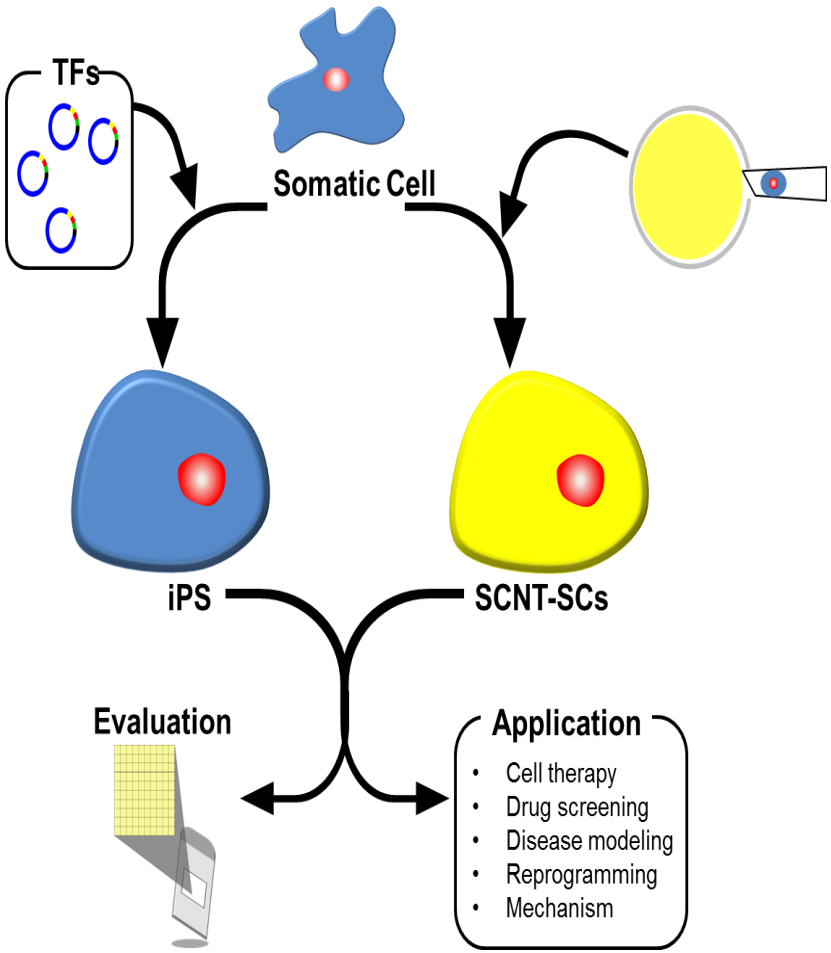
●: single mismatches at the two loci or better match

Japan., Homozygous iPS bank



Recent Animal Studies

➔ *Functional and Structural Comparison between Mouse SCNT- and iPSC derived–SC from Same Somatic Cells*



Cell Stem Cell
Article

Enhanced Telomere Rejuvenation in Pluripotent Cells Reprogrammed via Nuclear Transfer Relative to Induced Pluripotent Stem Cells

Rongrong Le,[1,2] Zhaohui Kou,[2] Yonghua Jiang,[1,2] Ming Li,[2] Bo Huang,[2] Wenqiang Liu,[1,2] Hui Li,[3] Xiaochen Kou,[2] Wanzhong He,[2] Karl Lenhard Rudolph,[4] Zhenyu Ju,[5,*] and Shaorong Gao[2,6,*]

Stem Cell Reports
Report
OPEN ACCESS

Mouse SCNT ESCs Have Lower Somatic Mutation Load Than Syngeneic iPSCs

Zhe Li,[1,3,4] Hongxia Lu,[2,4] Weifeng Yang,[2] Jun Yong,[2] Zhen-ning Zhang,[1] Kun Zhang,[3,*] Hongkui Deng,[2,*] and Yang Xu[1,*]
[1]Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0322, USA
[2]College of Life Sciences, Peking University, Beijing 100871, China
[3]Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0322, USA
[4]These authors contributed equally to this work
*Correspondence: kzhang@bioeng.ucsd.edu (K.Z.), hongkui_deng@pku.edu.cn (H.D.), yangxu@ucsd.edu (Y.X.)
http://dx.doi.org/10.1016/j.stemcr.2014.02.005

- **SCNT-mediated reprogramming mitigates telomere dysfunction and mitochondrial defects to a greater extent than iPSC-based reprogramming.**
- **When compared to induced pluripotency, SCNT might be a safer way to reprogram somatic cells into a pluripotent state with a lower mutation load.**

ARTICLE

doi:10.1038/nature13551

Abnormalities in human pluripotent cells due to reprogramming mechanisms

Hong Ma[1,2*], Robert Morey[3*], Ryan C. O'Neil[4,5], Yupeng He[4,5], Brittany Daughtry[1,2], Matthew D. Schultz[4], Manoj Hariharan[4], Joseph R. Nery[4], Rosa Castanon[4], Karen Sabatini[3], Rathi D. Thiagarajan[3], Masahito Tachibana[2†], Eunju Kang[1,2], Rebecca Tippner-Hedges[1,2], Riffat Ahmed[1,2], Nuria Marti Gutierrez[1,2], Crystal Van Dyken[1,2], Alim Polat[2†], Atsushi Sugawara[2], Michelle Sparman[2], Sumita Gokhale[6], Paula Amato[7], Don P. Wolf[2], Joseph R. Ecker[4,8], Louise C. Laurent[3] & Shoukhrat Mitalipov[1,2,7]

Ma et al., Nature 2014 (July)

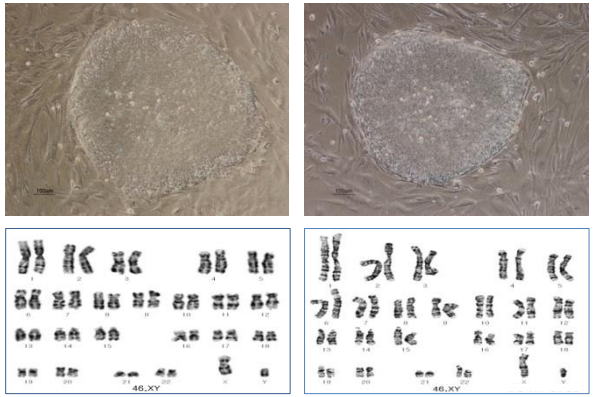
- Genetically matched cell lines (fetal dermal fibroblast, NT1-NT4, iPS-R1 and iPS-R2, iPS-S1-iPS-S5, hESO-7 and HESO-8) from 1 fetal skin fibroblast
- Subchromosomal aberrations
- Global DNA methylation, DNA methylation at imprinted and XCI regions
- Autosomal non-imprinted loci and whole-genome bisulphite sequencing
- Non-CG methylation in NT ES cells
- Global gene expression

- Both NT ES cells and iPS cells derived from the same somatic cells contained comparable numbers of de novo copy number variations.
- In contrast, DNA methylation and transcriptome profiles of NT ES cells corresponded closely to those of IVF ES cells, whereas iPS cells differed and retained residual DNA methylation patterns typical of parental somatic cells.

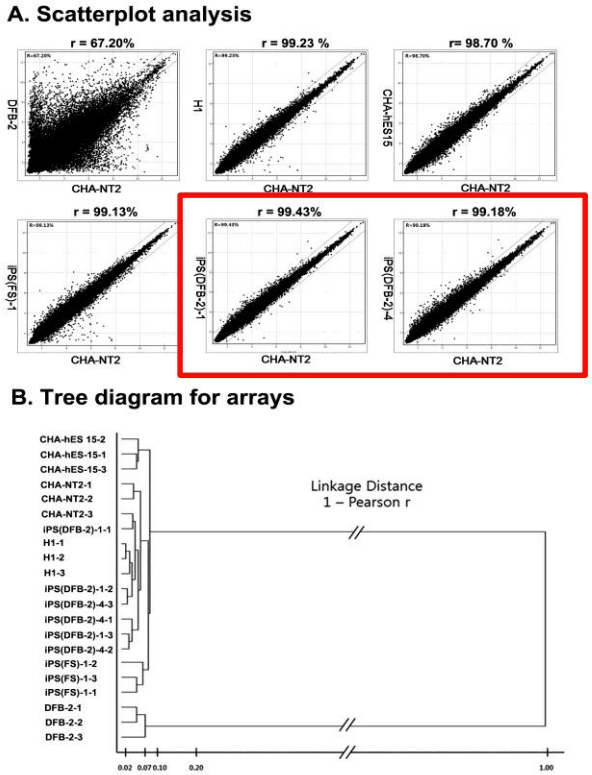
Recent studies

➔ Functional and Structural Comparison between Human SCNT- and iPSC derived–SC from Same Somatic Cells of Adult Patients

CHA-hES NT2 **CHA-hiPS NT2-S4**



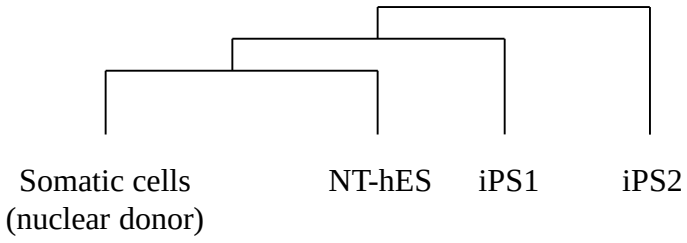
	Fibroblast (DFB-2)		CHA-hES NT2		CHA-hiPS-NT2-S4	
6-FAM_Blue						
D8S1179	14	16	14	16	14	16
D21S11	28	30	28	30	28	30
D7S820	9	10	9	10	9	10
CSF1PO	12	12	12	12	12	12
VIC_Green						
D3S1358	15	17	15	17	15	17
THO1	6	7	6	7	6	7
D13S317	9	11	9	11	9	11
D16S539	11	12	11	12	11	12
D2S1338	17	19	17	19	17	19
NED_Yellow						
D19S433	14	14	14	14	14	14
vWA	17	19	17	19	17	19
TPOX	9	10	9	10	9	10
D18S51	14	19	14	19	14	19
PET_Red						
AMEL	X	Y	X	Y	X	Y
D5S818	11	12	11	12	11	12
FGA	22	23	22	23	22	23



- Global expression profiles of SCNT-SC was very similar to those of same donor cells-derived iPSCs as well as conventional ESCs and iPSCs.
- Comparison of Genetic Stability between Human SCNT-SC and iPSC derived from Same Somatic Cells

Analysis of SNP variations from 3 sets of Pluripotent Stem Cells (1 NT-SC and 2 iPSCs)

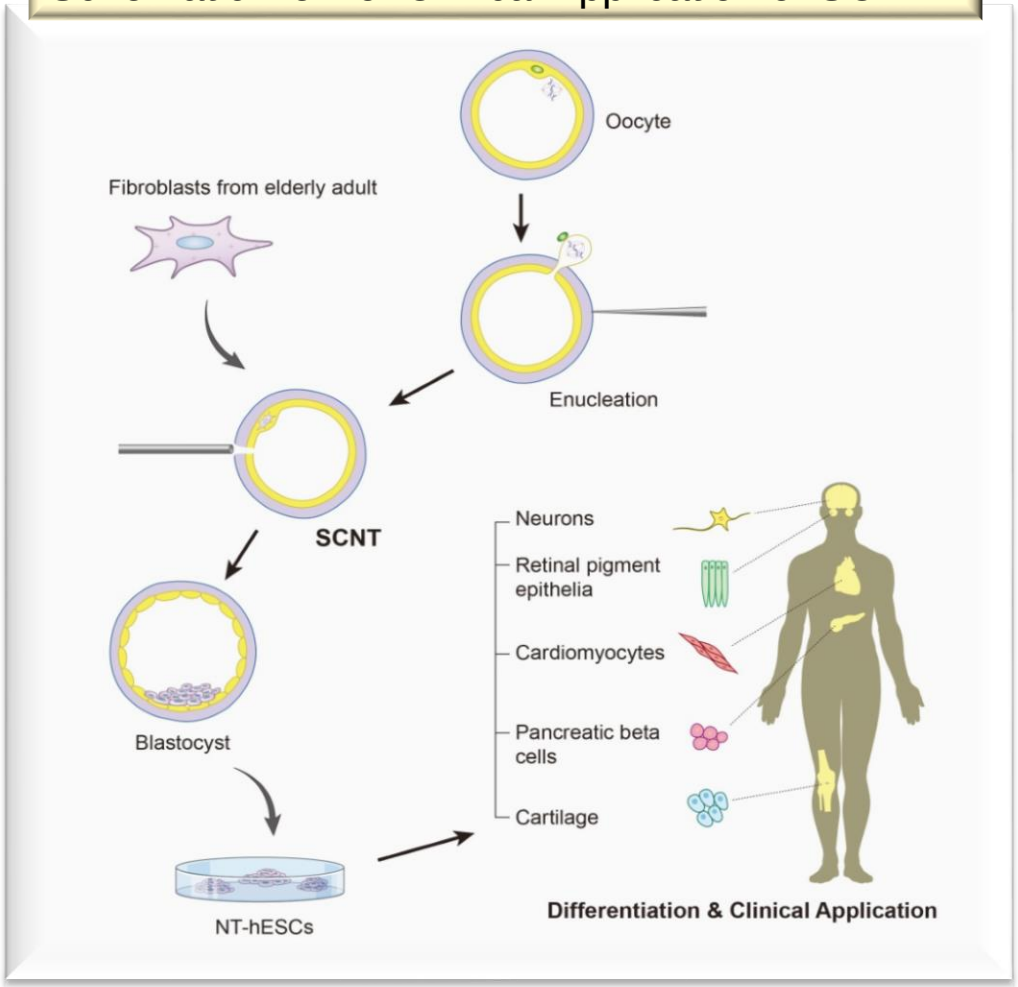
	Somatic cell (nuclear donor)	NT-SC	iPSC1	iPSC2
Group 1 (CHA-NT2)	641,995	**641,852(99.978%)**	641,758(99.963%)	641,848(99.977%)
Group 2 (CHA-NT4)	680,432	**680,384(99.993%)**	680,258(99.974%)	678,471(99.712%)
Group 3 CHA-NT5	668,528	668,245(99.958%)	668,136(99.941%)	**668,270(99.961%)**
Total	1,990,985	**1,990,481(99.975%)**	1,990,152(99.958%)	1,988,589(99.880%)



Unpublished data

Production of Patient-specific SCNT-RPE

Schematic view of Clinical Application of SCNT



Production of Patient specific SCNT-derived RPE

CHA Stem Cell Institute 2015

Stem Cell Team of CSCI
Kwang Yul Cha
Young Gie Chung
Sung Han Shim
Jin Hee Eum
Jeoung Eun Lee
Won-kyung Song

Collaboration
Robert Lanza (ACT Inc.)
Yi Zhang (Harvard Medical School)
Kwon-Ho Hong (Dankook Univ.)

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경청해 주셔서 감사합니다.