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Supplementary Material

In vitro culture of stem-like cells derived from somatic cell nuclear transfer bovine embryos of the Korean beef cattle species, HanWoo

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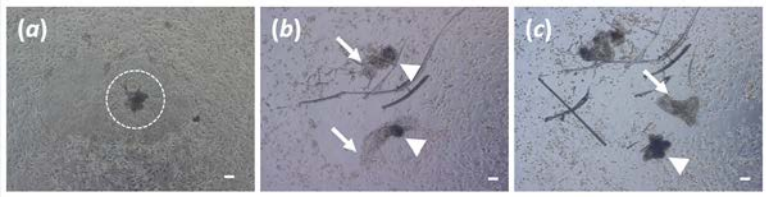

Fig. S1. The methods for passaging the central multilayer (CMt) cell clump in bovine somatic cell nuclear transfer embryo-derived stem-like cell (bSCNT-eSLC) colony. (A) Whole SCNT-eSLC colony. The dotted circle displays CMt part. (B) Split colony into two clumps which include both CMt (arrowhead) and peripheral monolayer (PMn, arrow) regions. (C) CMt was manually separated from PMn in the clump using a 26 gauge needle. Scale bar = 200 μm.

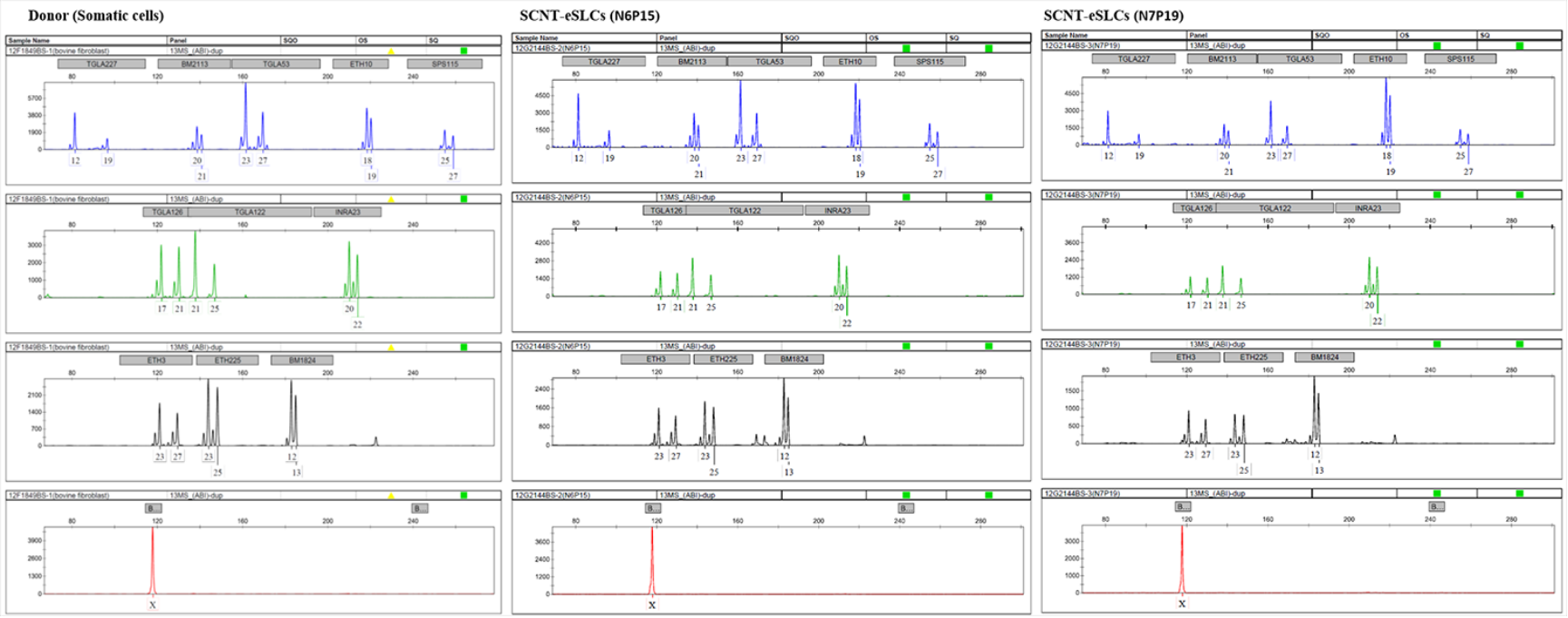

Fig. S2. Microsatellite DNA analyses of bovine somatic cell nuclear transfer embryo-derived stem-like cell (bSCNT-eSLC) colonies (N6P15 and N7P19; N6 line at passage 15 and N7 line at passage 19, respectively). The examination of eleven loci of TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, ETH3, ETH225 and BM1824 confirm that the bSCNT-eSLC colonies are genetically identical to the donor fetal fibroblasts used for nuclear transfer.

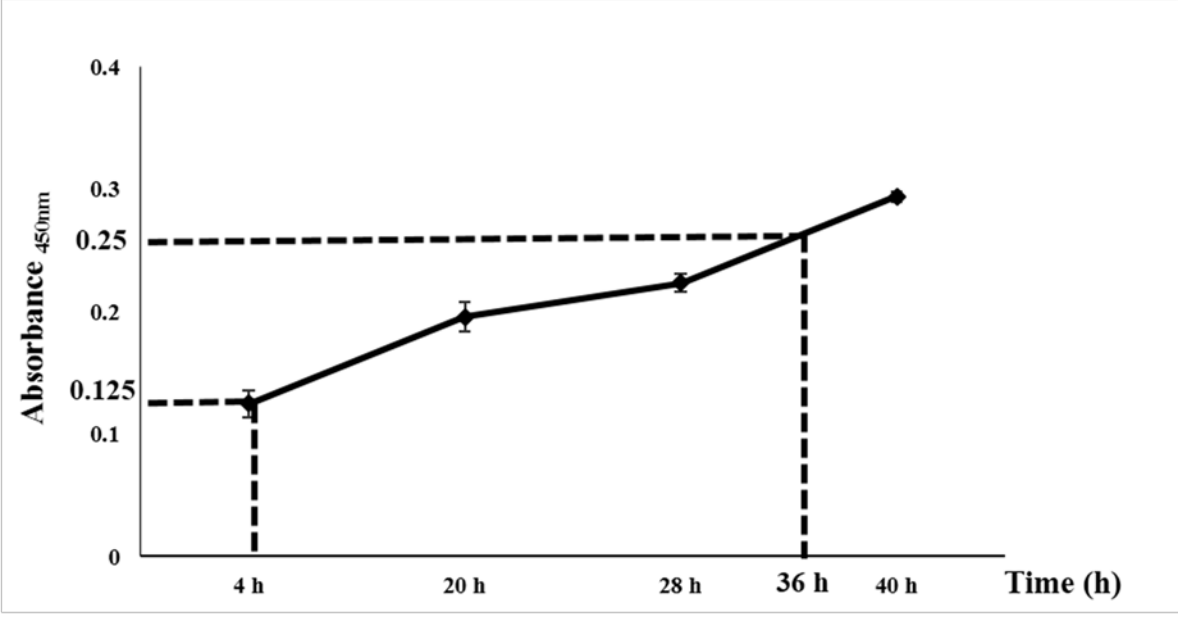

Fig. S3. Doubling time of bovine somatic cell nuclear transfer embryo-derived stem-like cells (bSCNT-eSLCs) during the culture. The absorbance (wave length = 450 nm) at 36 h was approximately double the absorbance at 4 h, indicating that the doubling time of bSCNT-eSLCs was about 32 h. N = 5.

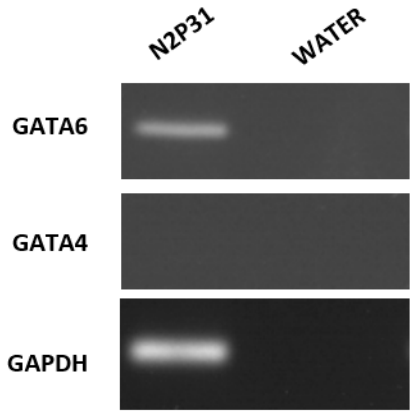

Fig. S4. Expression of hypoblast marker *GATA6* and *GATA4* in bovine somatic cell nuclear transfer embryo-derived stem-like cells (bSCNT-eSLCs) at P31 (N2P31). Among the hypoblast markers, only *GATA6* is expressed in bSCNT-eSLCs while *GATA4* and *α-Fetoprotein* (see Fig. 4*b*) are not.

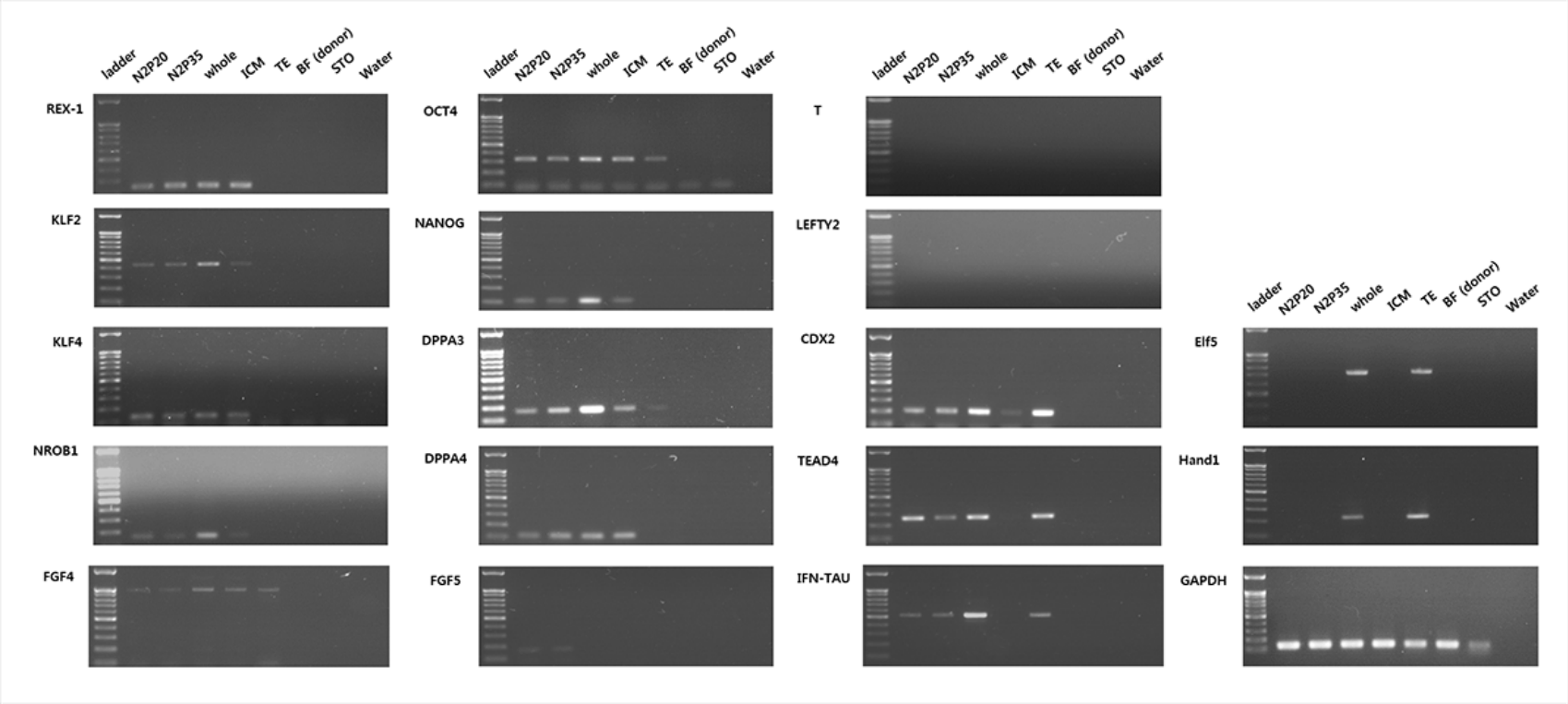

Fig. S5. The full size gels of RT-PCR results with DNA ladder as supplementary material to Fig. 4*c*. Expression of the pluripotency and trophectoderm specific marker genes by RT-PCR analysis in bSCNT-eSLCs at P20 (N2P20) and P35 (N2P35), inner cell mass (ICM), trophoblast (trophectodermal cell; TE), whole embryo (whole), STO (feeder) and bovine fibroblast (BF as nuclear donor). T = T-BRACHYURY.

Table S1. Generation of various bovine somatic cell nuclear transfer embryo-derived stem-like cell lines with 3i*

	Embryo source		
	In vitro fertilised	Parthenogenesis	Somatic cell nuclear transfer
Multi-embryo derived cell lines	11	7	7
Single-embryo derived cell lines	10	5	8

*PD184352 + CHIR99021 + SU5402

Table S2. Teratoma-like tumor formation after transplantation of bSCNT-eSLCs*

	No. of	
	Injection under testis capsule	*Teratoma-like tumor formation*
bSCNT-eSLCs	10	1 (embryonic carcinoma)
STO (feeder cells)	6	0

* Bovine somatic cell nuclear transfer embryo-derived stem-like cells

Table S3. DNA profile for bovine derived teratoma-like cells

Locus[a]	*Allele 1*[b]	*Allele 2*[b]
TGLA227	85.52	85.52
BM2113	140.51	140.51
TGLA53	171.45	180.00
ETH10	212.53	220.19
SPS115	247.16	249.08
TGLA126	121.82	121.82
TGLA112	148.80	148.80
IRA23	204.55	227.95
ETH3	117.67	127.09
ETH225	143.61	147.92
BM1824	187.10	195.16

[a]Bovine specific loci

[b]Reverse Transcription-Polymerase Chain Reaction product size

Table S4. Comparison of cell source for outgrowth of embryonic cells*

	Inner cell mass	*Trophectoderm*	*Whole embryo*
Maximum survival passage number	4	4	15 and over

*Three replicates; all the cases were conducted with 3i system.

Table S5. The survival rate of CMt- and PMn-derived colonies[a)]

			No. of survived colonies at			
		Group[b)]	*Passage 1*	*Passage 2*	*Passage 3*	*Passage 4*
Passaging the initial stage of colonies[c)]	CMt	C1	1	3	10	29
		C2	1	2	6	18
		C3	1	2	4	8
		C4	1	2	6	16
		C5	1	2	5	15
		Total (%)	5 (100.0)	11 (55.0)	31 (70.4)	86 (69.3)
	PMn	P1	1	2	1	0
		P2	1	2	2	0
		P3	1	0	0	0
		P4	1	1	0	0
		P5	1	0	0	0
		Total (%)	5 (100.0)	5 (50)	3 (25)	0 (0)

			No. of survived colonies at		
		Group[b)]	*Passage 22*	*Passage 23*	*Passage 24*
Passaging the proliferating stage of colonies[d)]	CMt	C1	1	4	13
		C2	1	3	8
		C3	1	2	5
		C4	1	2	11
		C5	1	3	4
		Total (%)	5 (100)	14 (70)	41 (73.2)
	PMn	P1	1	0	0
		P2	1	0	0
		P3	1	1	0
		P4	1	0	0
		P5	1	1	0
		Total (%)	5 (100)	2 (20)	0 (0)

[a)]CMt: central multilayer; PMn: peripheral monolayer.

[b)]Only one colony slice were used for the next passage. The CMt colony was sliced to 4 pieces while the PMn colony was sliced to 2 pieces due to the inability of attachment and proliferation of the cells in PMn colony in 3i culture condition.

c)Outgrown seeded blastocysts = Passage 0

[d)]Until passage 21, the stem cell colony was maintained only with CMt-derived cells. Up to passage 21, PMn part of the colony was discarded.