Characterization of hPSC

Cell Line Name	**hFmiPS1**
Type of Cell Line	**hiPSC**
Depositor (Institution)	**Korea National Institute of Health**
Passage #	**p26***
Day of Cell Freezing	**20140710**

Analysis	Result	Passage#	Day of analysis
Cell viability	Pass (78.1±8%)	p22	20140811
Authentication (STR)	Pass	p30	20140701
Mycoplasma test (PCR)	Pass	p23	20140528
Mycoplasma test (Luminometor)	Pass	p23	20140528
Cell attachment & colony morphology	Pass	p23	20130624
Microbial contamination test (Virus, Fungi, bacteria)	Pass	p26	20140612
Cell doubling time	19.9±3 hr	p26	20140701
Karyotype (G-banding)	46,XY,t(1:4)(q21q35)	p30	20140627
HLA genotype	HLA-A *02:07 *24:02g HLA-B *13:02 *35:01 HLA-DRB1 *01:01 *07:01	p30	20140704
ABO genotype	AA	p30	20140703
Stem Cell Marker Expression			
· AP staining	Pass (Positive)	p30	20140627
· ICC	Pass (Positive)	p25	20140811
· RT-PCR	Pass (Positive)	p25	20141103
· qRT-PCR	Pass (Positive)	p25	20141103
Differentiation Marker Expression			
· EB formation	Pass (EB14d)	p25	20140619
· RT-PCR	Pass (Positive)	p25	20141103
· qRT-PCR	Pass (Positive)	p25	20141103
· Teratoma formation	Pass (Three-germ layer)	p20	20141017

* Freezing media : mFreSR (Stem Cell Technol. #05855)

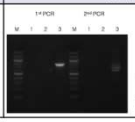
Cell Culture Condition	
· Feeder	STO (mouse embryonic fibroblast; ATCC CRC-1503)
· Media	hPS media (DMEM/F12 supplemented KSR and FGF2)
· Passage (Cell dissociation)	Dispase II (Gibco, #17105-041)
Description of the hPSC	
· Parental Cell	human dermal fibroblast (ScienceCell, #2320)
· Reprogram	modified mRNA (mRNA Reprogramming Kit, Stemgent #00-0071) OCT4, SOX2, KLF4, LIN28, c-MYC

Reference
Uhm KO et al. Generation of human induced pluripotent stem cell lines from human dermal fibroblasts using a modified RNA system. Stem Cell Res 2017 Oct;24:148-150.

Mycoplasma contamination test

<PCR>

시험일자: 2014년 5월 28일

시료명	판정 (positive, negative or 재검사)
hFmiPS1 p23	 negative

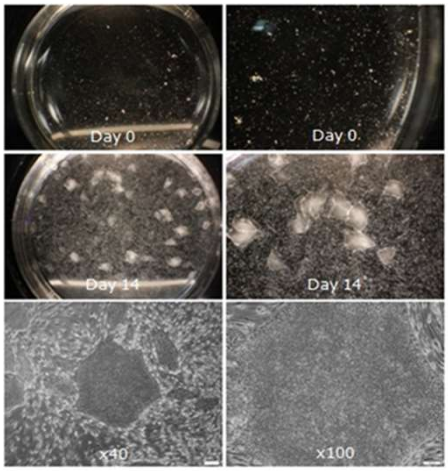
<루미노메터>

시험일자: 2014년 5월 28일

시료명	A 값	B 값	B/A 비율†	판정 (positive, negative or 재검사)
hFmiPS1 p23	2451	1210	0.49	Negative
NC	9257	587	0.06	Negative
PC	4581	169524	37.01	Positive

† < 0.9 Negative 마이코플라스마 미검출
> 1.2 Positive 마이코플라스마 오염
> 0.9~1.2 Borderline 재시험

Cell attachment and morphology

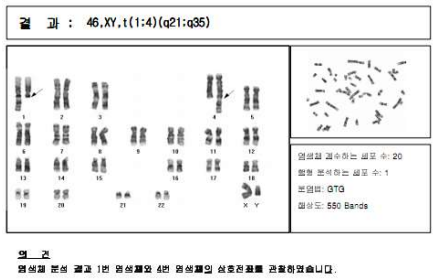


Microbial contamination test

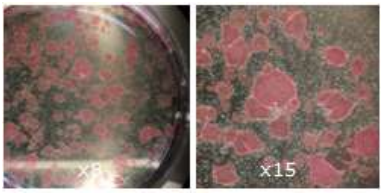
No	Test	References	hFmiPS1(p26)
1	HBV DNA 정량	**	**
	Copies/mL	<116	<116
	IU/mL	<20	<20
	pg/mL	<0.0004	<0.0004
2	HCV RNA 정량	**	**
	IU/mL	≤15	<15
	Copies/mL	≤40	<40
3	HIV RNA 정량	<20	<20
4	HTLV I& II Ab	Negative	Negative
5	EBV PCR	Negative	Negative
6	CMV PCR	Negative	Negative
7	HPV DNA 정량(Type16,Type18)	**	**
	High-risk HPV		Negative
	HPV Viral load		0.26
8	HSV Type I PCR	Negative	Negative
	HSV Type II PCR	Negative	Negative
	HSV Type I&II PCR	Negative	Negative
9	HHV Type 6 PCR	Negative	Negative
10	Culture & ID	No growth	No growth
11	Fungus Culture	No growth	No growth

Karyotype

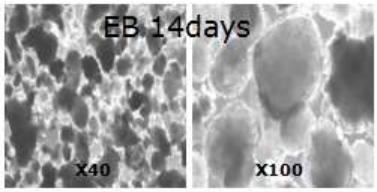
46,XY,t(1:4)(q21q35)



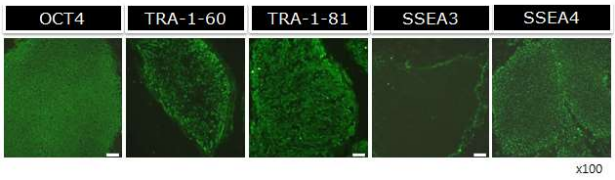
AP staining



EB formation



Stem cell marker gene expression



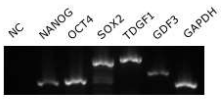
Teratoma formation

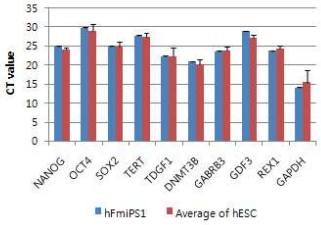
Stem cell marker gene expression

<qRT-PCR>

GENE	CTmean
NANOG	24.87
OCT4	29.86
SOX2	24.92
TERT	27.81
TDGF1	22.31
DNMT3B	20.92
GABRB3	23.68
GDF3	28.96
REX1	23.73
ACTB	18.63
RN18S1	13.94
18S	13.78
GAPDH	14.03

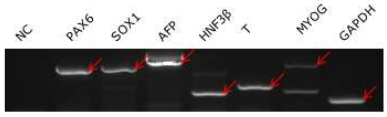
<RT-PCR>





Differentiation marker gene expression

<RT-PCR>



<qRT-PCR>

GENE	CTmean
PAX6	26.88
SOX1	22.89
HNF3B	30.64
AFP	35.89
T	28.90
MYOG	35.07
ATCB	18.88
RN18S1	14.43
18S	14.30
GAPDH	17.25