

Characterization of hPSC

Cell Line Name	CMC-003i-Nestin.EGFP			
Alternative Name	KSCBi005-A-1			
Type of Cell Line	hiPSC			
Depositor (Institution)	Korea National Institute of Health			
Passage #	p17*			
Day of Cell Freezing	20210521			
Analysis	Result	Passage #	Day of analysis	
Cell viability	Pass (Live cell# 9.6x10 ⁵ cell/mL)	p18	20211001	
Authentication (STR)	Pass	P19	20211020	
Mycoplasma test (PCR)	Pass	P15	20211014	
Cell attachment and colony morphology	Pass	p18	20211003	
Microbial test (Viral, bacterial, and fungal contamination)	Pass	p19	20211005	
Karyotype (G-banding)	46,XY	p15	20211005	
HLA (Genotype)	HLA-A *33:03 *33:03	p12	20180724	
	HLA-B *44:03 *44:03			
	HLA-DRB1 *13:02 *13:02			
ABO (Genotype)	AO	p8	20180713	
CNV analysis (CMA)	Pass (arr(X,Y)x1,(1-22)x2)	p17	20210709	
Stem Cell Marker Expression				
· AP staining	Pass (positive)	P18	20210525	
· ICC	Pass (positive)	p19	20210609	
· qRT-PCR	Pass (positive)	p19	20210608	
Differentiation Marker Expression				
· EB formation	Pass (EB14d)	p19	20210526	
· qRT-PCR	Pass (positive)	p19	20210724	

* Freezing media : Stem-cellbanker (Zenoaq) (Rec. : Use the ROCKi (e.g. Y27632) at cell thawing.)

Cell Culture Condition

- Feeder material · Vitronectin (Gibco, A14700)
- Media · TeSR-E8(Stem Cell Technol, ST05940) containing 150ng/ml Puromycin I
- Passage(Cell dissociation) · EDTA/Gentle Cell Dissociation Reagent (Stem cell Technol, 07174)

Cell Line Information

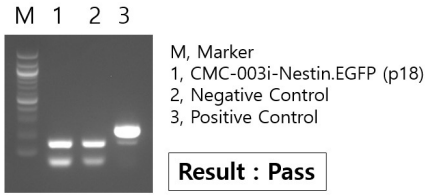
- Parental Cell · CMC-hiPSC-003 (Catholic University)
- Reprogramming · Method : Sendai virus (CytoTune-iPS Reprogramming kit, Invitrogen)
- Induction Genes : OCT3/4, SOX2, KLF4, c-MYC
- Genetic modification · CRISPR/Cas9- knock-in
- Nestin C-terminal(EGFP) Knock-in
- Puromycin Resistance

Reference

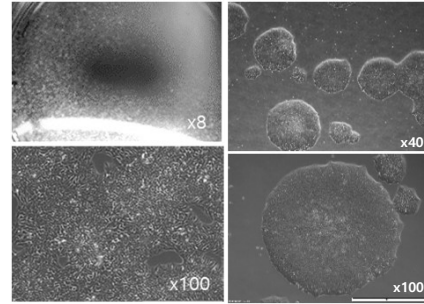
Lee Y et al. Generation of a NESTIN-EFGP reporter human induced pluripotent stem cell line, KSCBi005-A-1, using CRISPR/Cas9 nuclease. Stem Cell Res 2019 Oct;40:101554.

Mycoplasma contamination test

e-MtcoVALiD Mycoplasma PCR Detection Kit (iNtRON Biotech)



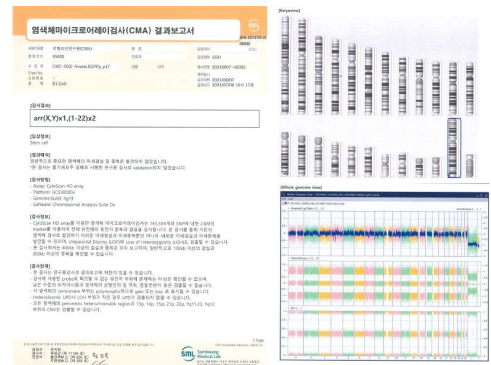
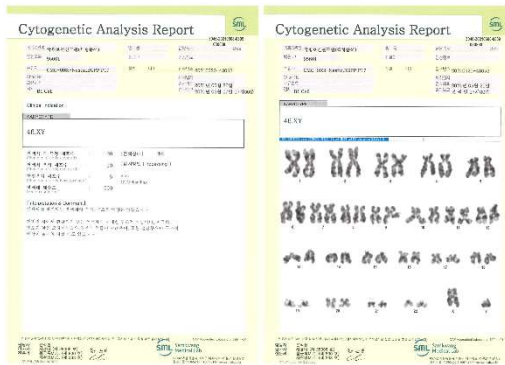
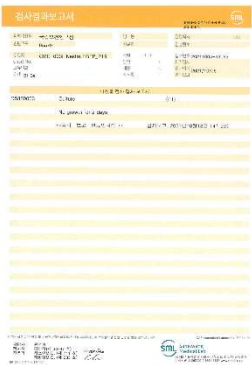
Cell attach and morphology



Microbial contamination test

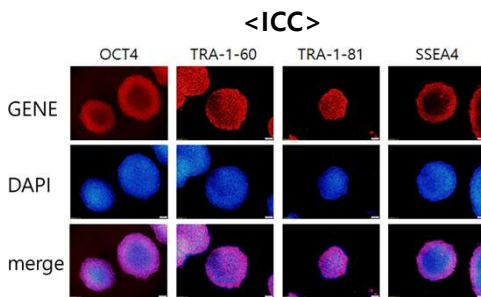
Karyotype

CNV analysis (CMA)



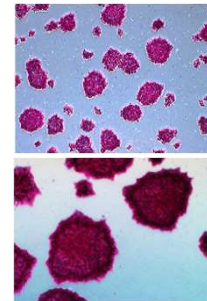
Stem cell marker gene expression

AP staining



<qRT-PCR>

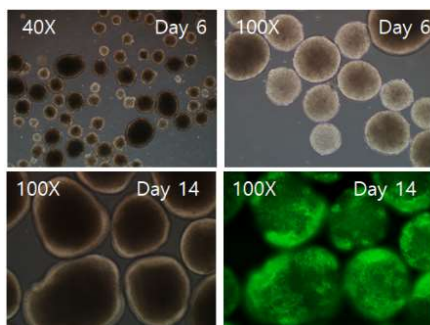
GENE	CT mean
NANOG	20.0
OCT4	26.6
SOX2	21.4
TERT	25.1
TDGF1	18.8
DNMT3B	19.9
GABRB3	20.0
GDF3	22.5
REX1	23.8
GAPDH	17.2



EB formation & Differentiation

Differentiation (Neuronal lineage)

<EB formation and GFP expression> <qRT-PCR, EB 14d>



GENE	CT mean
PAX6	23.2
NR2F2	28.1
EMA20S	27.6
T	25.2
HAND1	26.0
ITGA8	26.0
HNF3B	22.9
AFP	26.2
IHH	27.4
GAPDH	15.4

