Characterization of hPSC

Cell Line Name	**NCRM5AS-iCAGcGFP.c9**
Type of Cell Line	**hiPSC**
Depositor (Institution)	**NIH Center for Regenerative Medicine (US)**
Passage #	**p73***
Day of Cell Freezing	**20181004**

Analysis	Result	Passage #	Day of analysis
Cell viability	Pass (67.33±0.05 %)	p74	20181017
Authentication (STR)	pass	p74	20181030
Mycoplasma test (PCR)	Pass	p74	20181031
Cell attachment and colony morphology	Pass	p70	20180911
Karyotype (G-banding)	46,XY	p73	20181016
Stem Cell Marker Expression			
· AP staining	Pass	p71	20181018
· ICC	Pass	p71	20181018
· qRT-PCR	Pass	p71	20181011
Differentiation Marker Expression			
· EB formation, GFP expression	pass	p73	20181004
· qRT-PCR	pass	p74	20181026

* Freezing media   - Stem-Cell Banker GMP grade (Amsbio, Cat. 11897)
* Freezing method   - Controlled Rate Freezer
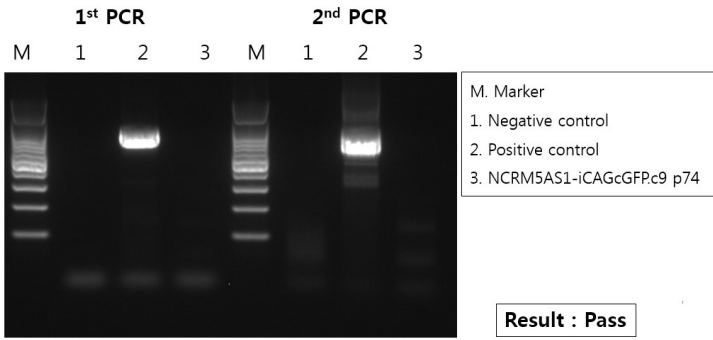
Cell Culture Condition

· Feeder/matrix	Vitronectin (Gibco, A14700)
· Media	TeSR-E8 (Stem Cell Technol, ST05940)
· Passage method	EZPassage(StemPro, 23181-010)

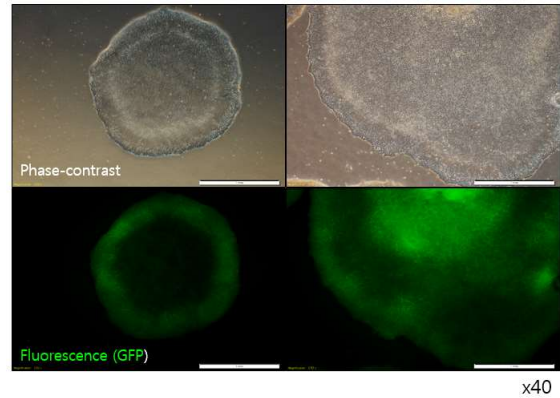## Description by depositor

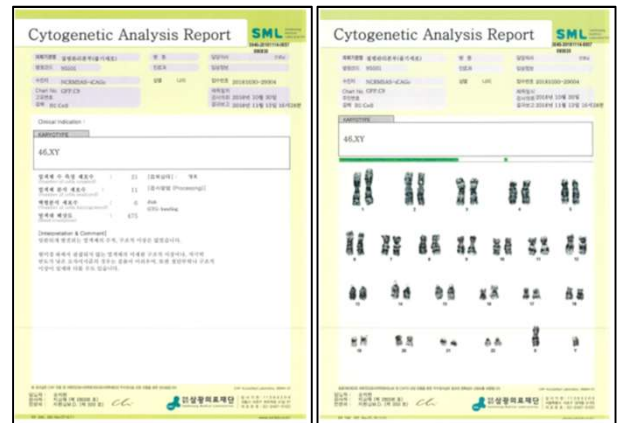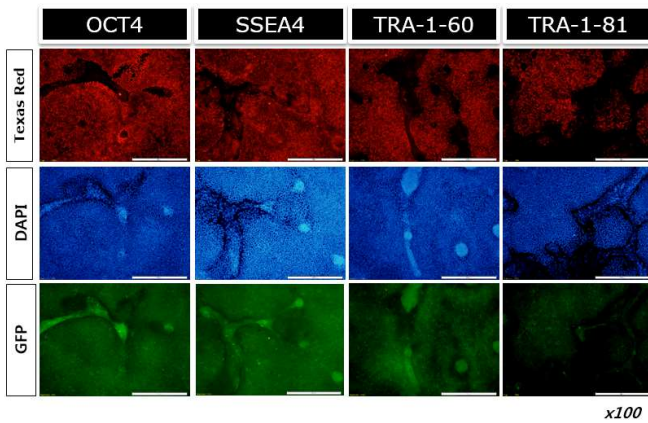|	
- Parental Cell	NCRM-5 (NCRM Cat#CR0000013)
	(CD34+ cord blood ; episomal plasmid)
- Genetic modification	GFP-report tagged; TALEN
- Reference	Luo et al., 2014. Stem Cell Translational Med (3) 821-835. Stable enhanced green fluorescent protein expression after differentiation and transplantation of reporter human induced pluripotent stem cells generated by AAVS1 transcription activator-like effector nucleases.

Mycoplasma contamination test

1st PCR   **2nd PCR**
M   1   2   3   M   1   2   3

M. Marker
1. Negative control
2. Positive control
3. NCRM5AS1-iCAGcGFP.c9 p74

Result : Pass

Cell morphology

Phase-contrast

Fluorescence (GFP)

x40

Microbial contamination test

Karyotype

46,XY

Stem cell marker gene and GFP expression

	OCT4	SSEA4	TRA-1-60	TRA-1-81
Texas Red				
DAPI				
GFP				

x100

AP staining

x10

EB formation

Day 7   Day 14   Day 14_GFP

x40
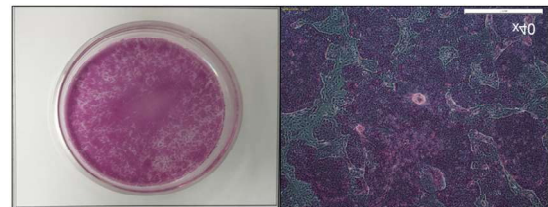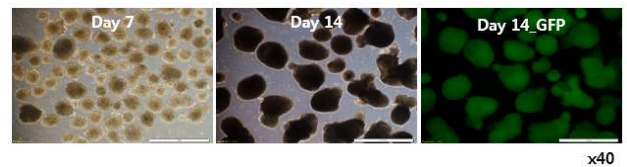
Stem cell marker gene expression

<qRT-PCR>

Gene	Ct Mean
NANOG	21.47
OCT4	26.31
SOX2	23.91
TERT	26.96
TDGF1	21.23
DNMT3B	20.89
GABRB3	21.70
GDF3	25.53
REX1	24.51
GAPDH	18.73

NCRM5AS1-iCAGcGFP.c9(MCB)

NANOG OCT4 SOX2 TERT TDGF1 DNMT3B GABRB3 GDF3 REX1

■ NCRM5AS1-iCAGcGFP.c9   ■ PCB BANK (n=30)
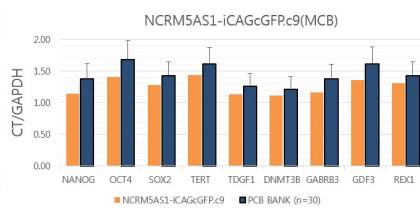
Differentiation marker gene expression (EB14d)

<qRT-PCR>

Gene	Ct Mean
PAX6	25.29
SOX1	31.75
HNF3B	23.75
AFP	22.54
T	27.92
MYOG	32.83
ACTB	18.33
GAPDH	19.30

NCRM5AS1-iCAGcGFP.c9 (MCB)

PAX6 SOX1 HNF3B AFP T MYOG

■ NCRM5AS1-iCAGcGFP.c9   ■ BANK (n=28)