

# Characterization of hPSC

<b>Cell Line Name</b>	<b>hiPSC-TLR2KO</b>		
Alternative name	CMC-hiPSC-003(TLR2KO)		
<b>Type of Cell Line</b>	<b>hiPSC</b>		
<b>Depositor (Institution)</b>	<b>Korea National Institute of Health</b>		
<b>Passage #</b>	<b>p35*</b>		
<b>Day of Cell Freezing</b>	<b>20200701*</b>		
Analysis	Result	Passage #	Day of analysis
Cell viability	Pass(81.1±4.8%)	p35	20210927
Authentication (STR)	Pass	p35	20210608
Mycoplasma test (PCR)	Pass	p35	20210622
Cell attachment and colony morphology	Pass	p35	20211001
Bacterial, and fungal contamination test	Pass	p35	20210611
Viral contamination test	Pass	p42	20210608
Karyotype (G-banding)	46,XY	p42	20210622
Stem Cell Marker Expression			
· AP staining	Pass (positive)	p31	20181026
· ICC	Pass (positive)	p32	20200707
· qRT-PCR	Pass (positive)	p31	20190121
Differentiation Marker Expression			
· EB fomatation	Pass (EB14d)	p35	20211018
· qRT-PCR	Pass (positive)	p31	20190121
· Hematopoietic/ macrophage lineage	Pass (positive)	p33	20190528

\* Freezing media : Stem-cellbanker (Zenoaq #BLC-3-1)

## Cell Culture Condition

- Feeder(matrix) : - Vitronectin (Gibco, A14700)
- Media : - Essential 8 (Gibco, ThermoFisher, A1517001)  
- Clone R (Stem Cell Technol., CAT#05888) or Y27632, at thawing
- Passage (Cell dissociation) - EDTA or Gentle Cell Dissociation Reagent (Stem cell Technol, 07174)  
- EZPassage (Thermo-Fisher 23181010)

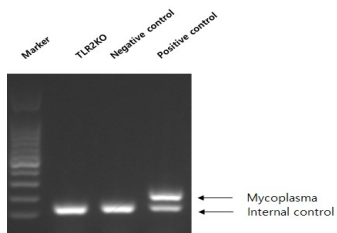
## Genetic Modification

- Parental Cell - CMC-hiPSC-003(Catholic University of Korea)
- Genetic modification - CRISPR/Cas9, TLR2 knock-out

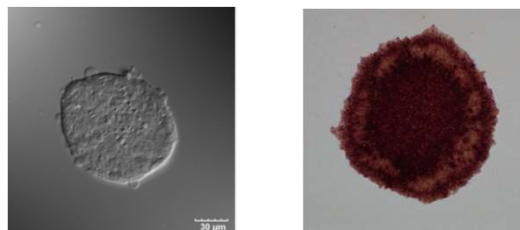
## Reference

Han HJ et al, Generation of a TLR2 knockout human induced pluripotent stem cell line using CRISPR/Cas9. Stem Cell Res. 2021 Oct 19;57:102578.

### Mycoplasma contamination test



### Cell Morphology AP staining



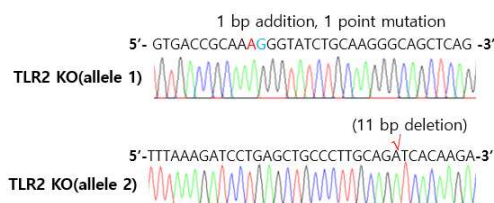
### Microbial contamination test



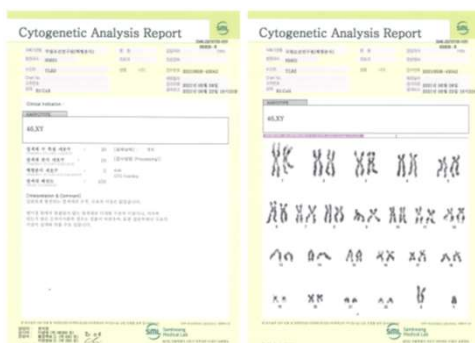
### TLR2 Knock-out (Sequencing)

Reference Allele 1: 5'-GTGACCGCAATGGTATCTGCAAGGGCAGCTCAG-3'  
 Allele 2: 3'-CACTGGCGTTACCATAGACGTTCCCGTCGAGTC-5'

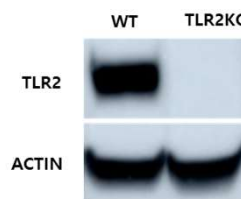
TLR2 KO Allele 1: 5'-GTGACCGCAAAGGGTATCTGCAAGGGCAGCTCAG-3'  
 Allele 2: 3'-CACTAGACGTTCCCGTCGAGTC-5'



### Karyotype

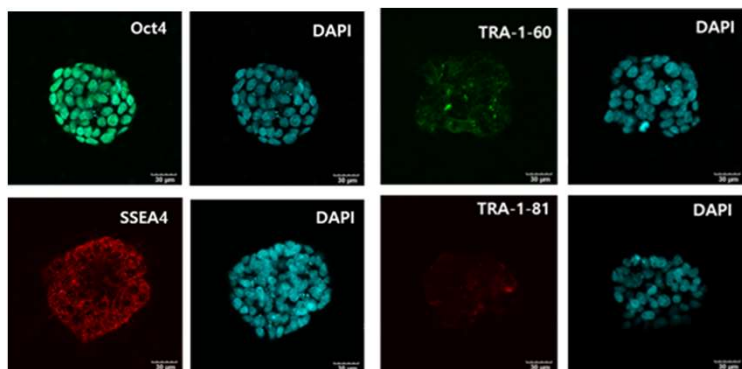


### TLR2 Expression in hematopoietic cells (macrophage)



### Stem cell marker gene expression

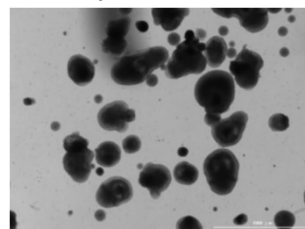
<ICC>>



### Differentiation

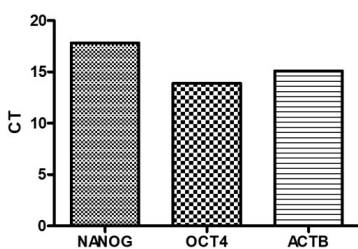
<EB formation>

EB 14 day



<qRT-PCR>

유전자	결과 (CT mean)
NANOG	17.8
OCT4	13.8
ACTB	15.0



<qRT-PCR>

유전자	결과 (CT mean)
ACTB	15.4
PAX6	26.2
SOX17	24.2
AFP	22.2
T	23.9
HAND1	16.5

