

Characterization of hPSC

Cell Line Name	hiPSC-TLR3KO-A49		
Alternative name	CMC-hiPSC-003(TLRKO-A10)		
Type of Cell Line	hiPSC		
Depositor (Institution)	Korea National Institute of Health		
Passage #	p39*		
Day of Cell Freezing	20200630*		
Analysis	Result	Passage #	Day of analysis
Cell viability	Pass(81.2±5.3%)	p39	20210927
Authentication (STR)	Pass	p39	20210608
Mycoplasma test (PCR)	Pass	p39	20211005
Cell attachment and colony morphology	Pass	p39	20211001
Bacterial, and fungal contamination test	Pass	p39	20210628
Viral contamination test	Pass	p39	20210611
Karyotype (G-banding)	46,XY	p39	20210622
Stem Cell Marker Expression			
· AP staining	Pass (positive)	p34	20181026
· ICC	Pass (positive)	p33	20200707
· qRT-PCR	Pass (positive)	p34	20181113
Differentiation Marker Expression			
· EB fomation	Pass (EB14d)	p39	20211018
· qRT-PCR	Pass (positive)	p34	20181113
· Hematopoietic/ macrophage lineage	Pass (positive)	p36	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)

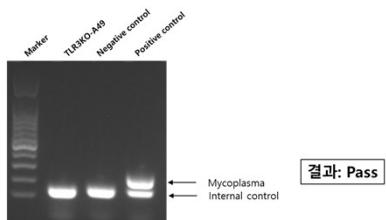
Genetic Modification

- Parental Cell - CMC-hiPSC-003(Catholic University of Korea)
- Genetic modification - CRISPR/Cas9, TLR8 knock-out

Reference

Han HJ et al., Establishment of a TLR3 homozygous knockout human induced pluripotent stem cell line using CRISPR/Cas9. Stem Cell Res. 2021 Apr;52:102187. doi: 10.1016/j.scr.2021.102187.

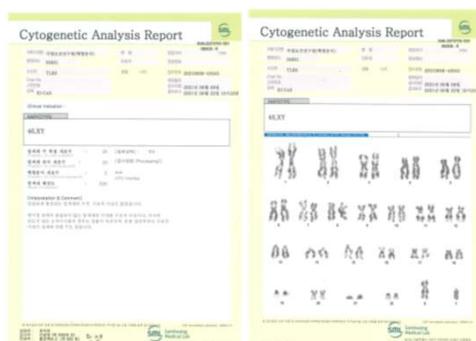
Mycoplasma contamination test



Microbial contamination test

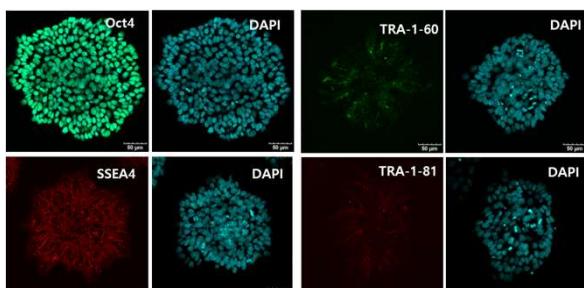


Karyotype

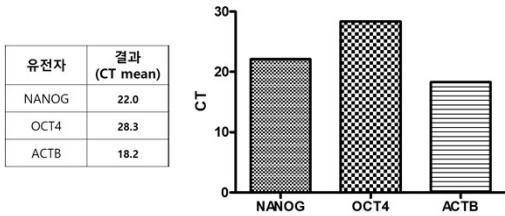


Stem cell marker gene expression

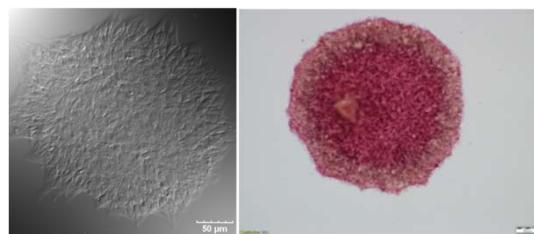
<ICC>>



<qRT-PCR>

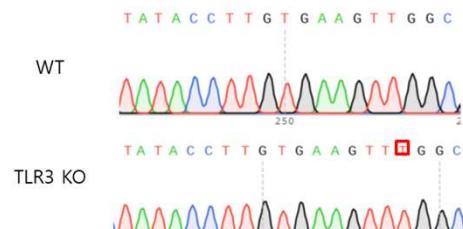


Cell Morphology AP staining

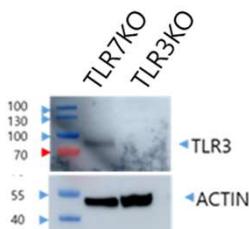


TLR3 Knock-out (Sequencing)

Reference 5'-TATACCTTGTGAAGTTGG-3'
TLR3 KO Line 5'-TATACCTTGTGAAGTTGG-3' 1bp addition

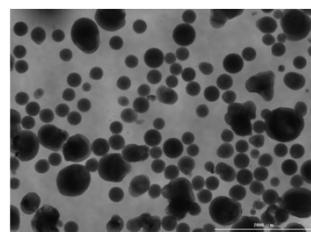


TLR3 Expression in hematopoietic cells (macrophage)



Differentiation <EB formation>

EB 14 day



<qRT-PCR>

