

# Characterization of hPSC

<b>Cell Line Name</b>	<b>PB01-EiPS21(GFP-NLS7)</b>		
<b>Type of Cell Line</b>	<b>hiPSC</b>		
<b>Depositor (Institution)</b>	<b>Korea National Institute of Health</b>		
<b>Passage #</b>	<b>p43*</b>		
<b>Day of Cell Freezing</b>	<b>20221121</b>		
<b>Analysis</b>	<b>Result</b>	<b>Passage #</b>	<b>Day of analysis</b>
Cell viability	Pass(32.7±1.0%) Live cell# : 5.83x10 <sup>5</sup> cell/ml	p45	20221123
Authentication (STR)	Pass	p46	20221209
Mycoplasma test (PCR)	Pass	p45	20221130
Cell attachment and colony morphology	Pass	p45	20221129
Bacterial, and fungal contamination test	Pass	p46	20221224
Viral contamination test	Pass	p46	20221208
Karyotype (G-banding)	46,XY	p46	20221220
Stem Cell Marker Expression			
· AP staining	Pass (positive)	p43	20221123
· ICC	Pass (positive)	p45	20221205
· qRT-PCR	Pass (positive)	p46	20221207
Differentiation Marker Expression			
· EB formation	Pass (EB14d)	p44	20221129
· qRT-PCR	Pass (positive)	p44	20221208
GFP Expression	Pass (positive)	p44	20221110

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

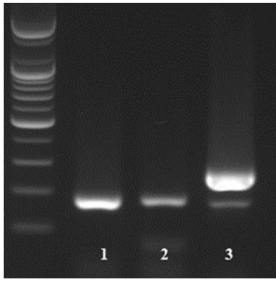
## Cell Culture Condition

- Feeder(matrix) : - Vitronectin (Gibco, A14700)
- Media : - StemFlex (Gibco/Thermo-Fisher A3349401)
- Passage (Cell dissociation) - EDTA or Gentle Cell Dissociation Reagent (Stem cell Technol, 07174)  
- EZPassage (Thermo-Fisher 23181010)

## Genetic Modification

- Parental Cell - PB01-EiPSC21 (Korea National Institute of Health)  
PBMC
- Genetic modification - CRISPR/Cas9, EGFP knock-in, AAVS1 locus

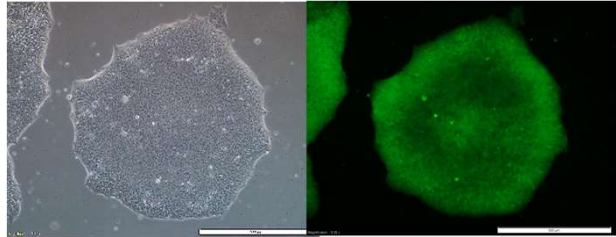
### Mycoplasma contamination test



- 1. Negative control
- 2. PB01-EiPS21(GFP-NLS7)
- 3. Positive control

### Cell attachment, Morphology & GFP expression

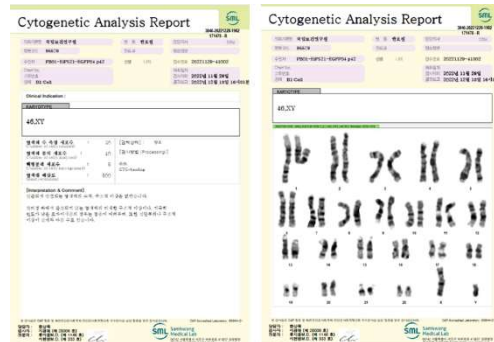
<Phase-contrast>      <Fluorescence>



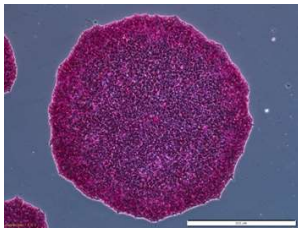
### Microbial contamination test



### Karyotype



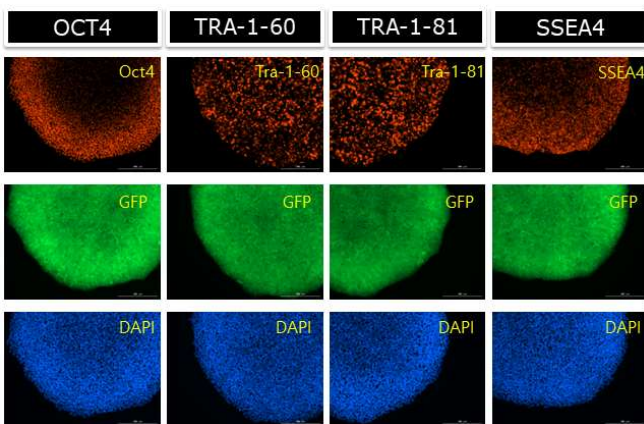
### AP staining



- 1. Negative control
- 2. PB01-EiPS21(GFP-NLS7)
- 3. Positive control

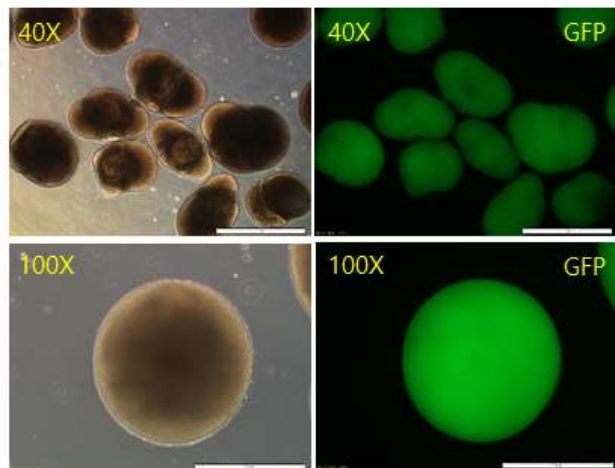
### Stem cell marker gene expression

<ICC>>



### Differentiation

EB 14day

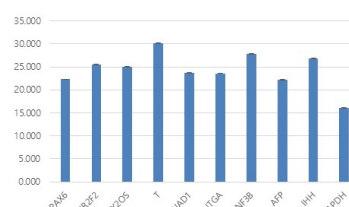


<qRT-PCR>

Gene	Ct mean
Nanog	23.999
OCT4	29.758
Sox2	23.962
TERT	27.733
TDGF1	22.377
DNMT3B	21.854
GABRB3	23.782
GDF3	27.833
REX1	24.046
GAPDH	19.657



<qRT-PCR>



Gene	Ct mean
PAX6	22.282
NR2F2	25.528
EMX2OS	25.056
T	30.187
HAND1	23.727
ITGA8	23.603
HNF3B	27.764
AFP	22.124
IHH	26.868
GAPDH	16.031