

Information Summary

Organization: Neural Stem Cell Institute (NSCI) **Address:** One Discovery Drive, Rensselaer, NY 12144

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SCRC Code: NSCI4

Sample Name: GIH-36

Number of the Established iPSC Clones: 6

Characterization Performed:

1. Mycoplasma Test

2. Cell authentication Verification

3. Presence of the Pluripotency Marker

4. Spontaneous differentiation via embryoid body (EB) formation

5. Karyotyping

Qualified iPSC Clones: C1, C2, C3



Mycoplasma Test

Method: Determination of the mycoplasmal enzyme activity using Lonza MycoAlert Plus Detection kit.

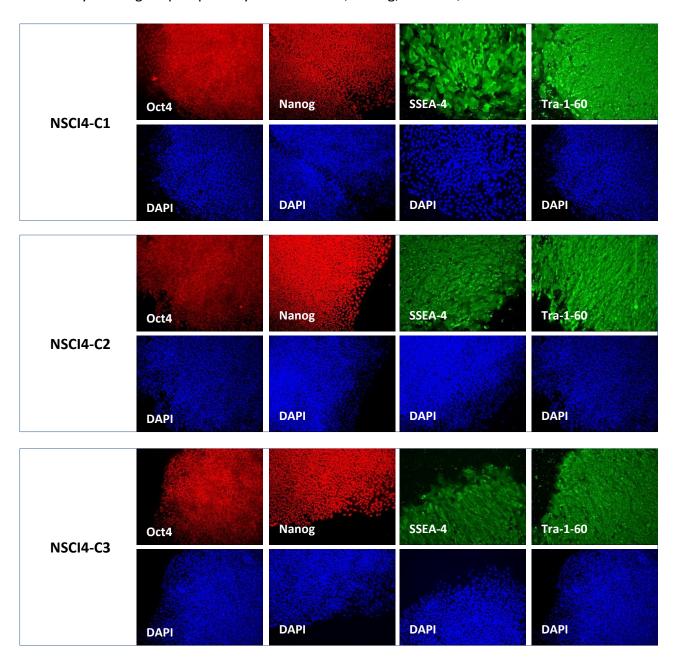
iPSC Clone	NSCI4-C1	NSCI4-C2	NSCI4-C3
Cell passage	Р6	P6	P6
B/A Ratio	0.62	0.57	0.61
Mycoplasma	NEG	NEG	NEG
Pass or Fail	Pass	Pass	Pass

Cell Authentication Verification

Markers	NSCI4 (GIH-36)	NSCI4-C1	NSCI4-C2	NSCI4-C3
D3S1358	15, 18	15, 18	15, 18	15, 18
TH01	7, 9	7, 9	7, 9	7, 9
D21S11	29, 31.2	29, 31.2	29, 31.2	29, 31.2
D18S51	13, 15	13, 15	13, 15	13, 15
Penta E	7	7	7	7
D5S818	12	12	12	12
D13S317	8, 12	8, 12	8, 12	8, 12
D7S820	11	11	11	11
D16S539	11, 13	11, 13	11, 13	11, 13
CSF1PO	9, 12	9, 12	9, 12	9, 12
Penta D	12	12	12	12
vWA	16, 18	16, 18	16, 18	16, 18
D8S1179	10, 11	10, 11	10, 11	10, 11
TPOX	11	11	11	11
FGA	20, 21	20, 21	20, 21	20, 21
AMEL	Χ, Υ	X, Y	X, Y	X, Y
Match with original Fibroblast		Yes	Yes	Yes

Expression of the Pluripotency Markers

Antibody staining for pluripotency markers: Oct4, Nanog, Tra-1-81, SSEA-4





Embryoid Body (EB) Formation

Method: The iPSC cells were disassociated into small clumps to form EB in the low attachment dish. After a 10-day spontaneous differentiation, the EBs were harvested and RNAs were extracted. The expression levels of the selected 3-germ layer specific genes were analyzed by real-time PCR. Ct values are normalized for loading using a housekeeping gene.

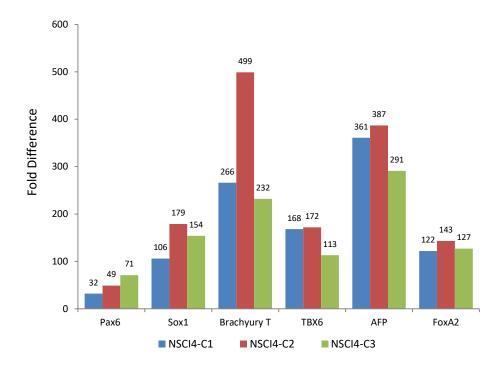


Figure: Lineage specific gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell.



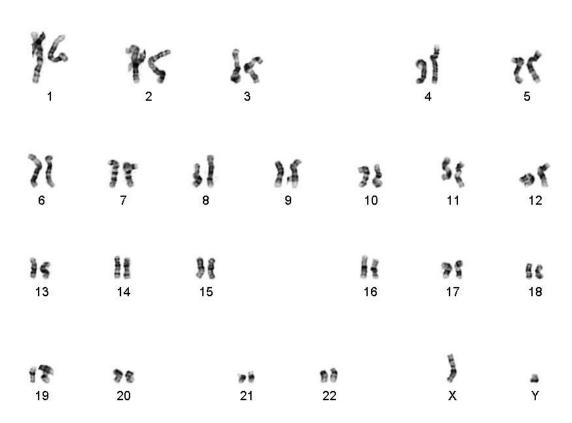
Karyotyping

Clone: NSCI4-C1 Passage: P7

Tested: Number of metaphases counted: 20

Number of metaphases analyzed: 6 Number of karyotypes: 2

Result: 46,XY NORMAL MALE KARYOTYPE



Banding and level: GTG/450



Karyotyping

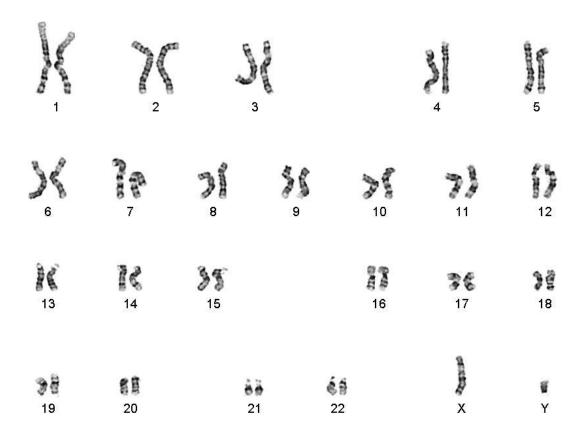
Clone: NSCI4-C2 Passage: P7

Tested: Number of metaphases counted: 20

Number of metaphases analyzed: 6

Result: 46,XY NORMAL MALE KARYOTYPE

Banding and level: GTG/500 Number of karyotypes: 2





Karyotyping

Clone: NSCI4-C3 Passage: P7

Tested: Number of metaphases counted: 20

Number of metaphases analyzed: 6

46,XY NORMAL MALE KARYOTYPE Result:

Banding and level: GTG/525 Number of karyotypes: 2

