

#### **Information Summary**

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SCRC Code: NSCI2

Sample Name: GIH-169

#### 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	NSCI2-C1	NSCI2-C2	NSCI2-C3
Cell passage	P6	P6	P6
B/A Ratio	0.23	0.75	0.70
Mycoplasma	NEG	NEG	NEG
Pass or Fail	Pass	Pass	Pass

## **Cell Authentication Verification**

Markers	NSCI2 (GIH-169)	NSCI2-C1	NSCI2-C2	NSCI2-C3
D3S1358	15	15	15	15
TH01	6, 7	6, 7	6, 7	6, 7
D21S11	27, 28	27, 28	27, 28	27, 28
D18S51	14, 17	14, 17	14, 17	14, 17
Penta E	7, 12	7, 12	7, 12	7, 12
D5S818	12, 13	12, 13	12, 13	12, 13
D13\$317	11, 12	11, 12	11, 12	11, 12
D7S820	10	10	10	10
D16S539	9, 12	9, 12	9, 12	9, 12
CSF1PO	12	12	12	12
Penta D	9, 12	9, 12	9, 12	9, 12
vWA	17, 18	17, 18	17, 18	17, 18
D8S1179	11, 13	11, 13	11, 13	11, 13
ΤΡΟΧ	8	8	8	8
FGA	24	24	24	24
AMEL	х	х	х	х
Match with orig	Match with original Fibroblast		Yes	Yes



## Expression of the Pluripotency Markers

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

NSCI2-C1	Oct4	Nanog	SSEA-4	Tra-1-60
	DAPI	DAPI	DAPI	DAPI
NSCI2-C2	Oct4	Nanog	SSEA-4	Tra-1-60
	DAPI	DAPI	DAPI	DAPI
NSCI2-C3	Oct4	Nanog	SSEA-4	Tra-1-60
	DAPI	DAPI	DAPI	DAPI

#### **Embryoid Body (EB) Formation**

Method: The iPSC cells were disassociated into small clumps to form EB in the low attachment dish. After a 10day 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.

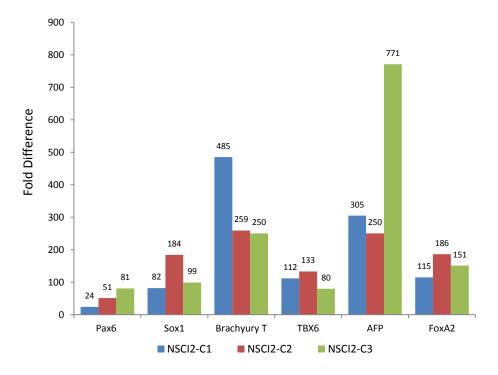


Figure: Lineage specific gene expression following EB differentiation. Fold difference is shown relative to undifferentiated iPS cell.



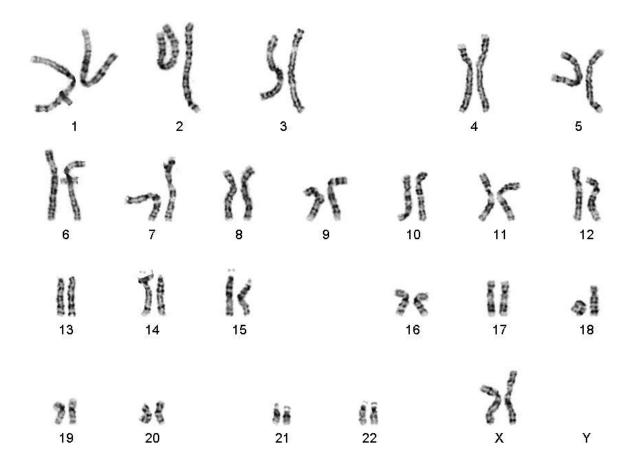
### Karyotyping

Clone: NSCI2 (GIH-169)-C1 Passage: P7

Tested:Number of metaphases counted: 20<br/>Number of metaphases analyzed: 6

Banding and level: GTG/550 Number of karyotypes: 2

**Result:** 46,XX NORMAL FEMALE KARYOTYPE





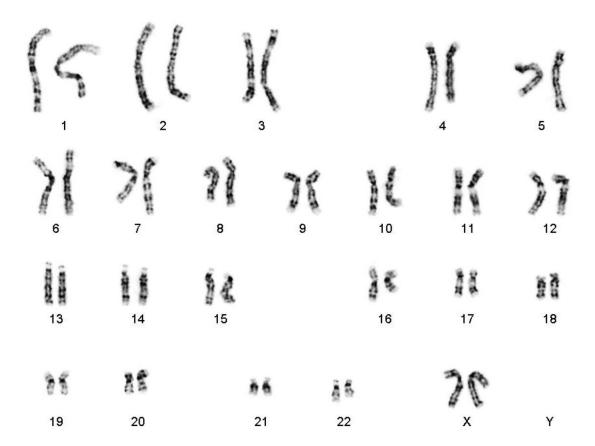
### Karyotyping

Clone: NSCI2 (GIH-169)-C1 Passage: P7

Tested:Number of metaphases counted: 20Number of metaphases analyzed: 6

Banding and level: GTG/450 Number of karyotypes: 2

**Result:** 46,XX NORMAL FEMALE KARYOTYPE





### Karyotyping

Clone: NSCI2 (GIH-169)-C3 Passage: P7

Tested:Number of metaphases counted: 20<br/>Number of metaphases analyzed: 6

Banding and level: GTG/550 Number of karyotypes: 2

**Result:** 46,XX NORMAL FEMALE KARYOTYPE

