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HIGHLY EFFICIENT AND ROBUST GENERATION OF NEURAL STEM CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING A SERUM-FREE NEURAL INDUCTION MEDIUM

Yiping Yan[1], Soojung Shin[1], Balendu Shekhar Jha[2], Jianting Shen[3], Ming Zhan[3], Sams Alexandria[1], David Kuninger[1], Mahendra Rao[2], Nasir Malik[2] and Mohan C Vemuri[1]

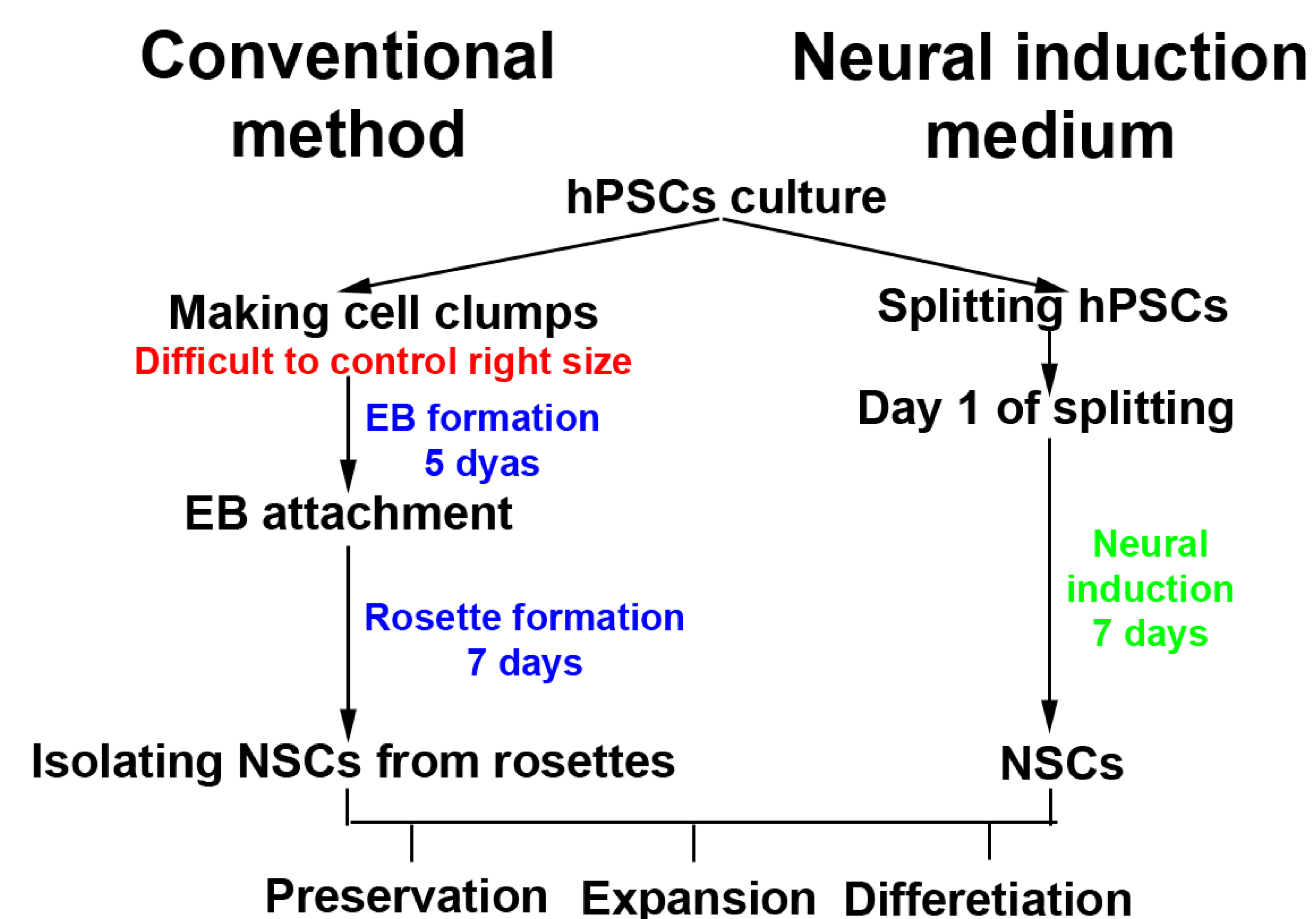
[1]Primary & Stem Cell Systems, Life Technologies, 7335 Executive Way, Frederick, MD 21704.
[2]National Center of Regenerative Medicine, National Institutes of Health, Bethesda, Maryland, USA
[3]The Methodist Hospital Research Institute, Houston, TX. 77030, USA

INTRODUCTION

Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) are excellent sources for studies of cell fate specification, disease modeling and drug screening. In order to produce various neural cells from hPSCs, the induction to neural stem cells (NSCs) is the first important step. Conventional methods of NSC derivation from hPSCs typically involve in embryoid body (EB) formation or co-cultures with stromal cell lines, which have several disadvantages such as time-consuming and multi-procedures, low efficiency and variable quality of derived NSCs. There is a critical need to develop a medium that will be easy to use and scalable to enable large scale generation of NSCs. We have developed a serum-free neural induction medium (Cat. no. A15640SA) which can convert hPSCs into NSCs in one week but without the time consuming, laborious processes of EB formation and mechanical NSC isolation.

RESULTS

1. Comparison of NSC derivation work flow between conventional method and neural induction medium



2. Cell morphology during neural induction

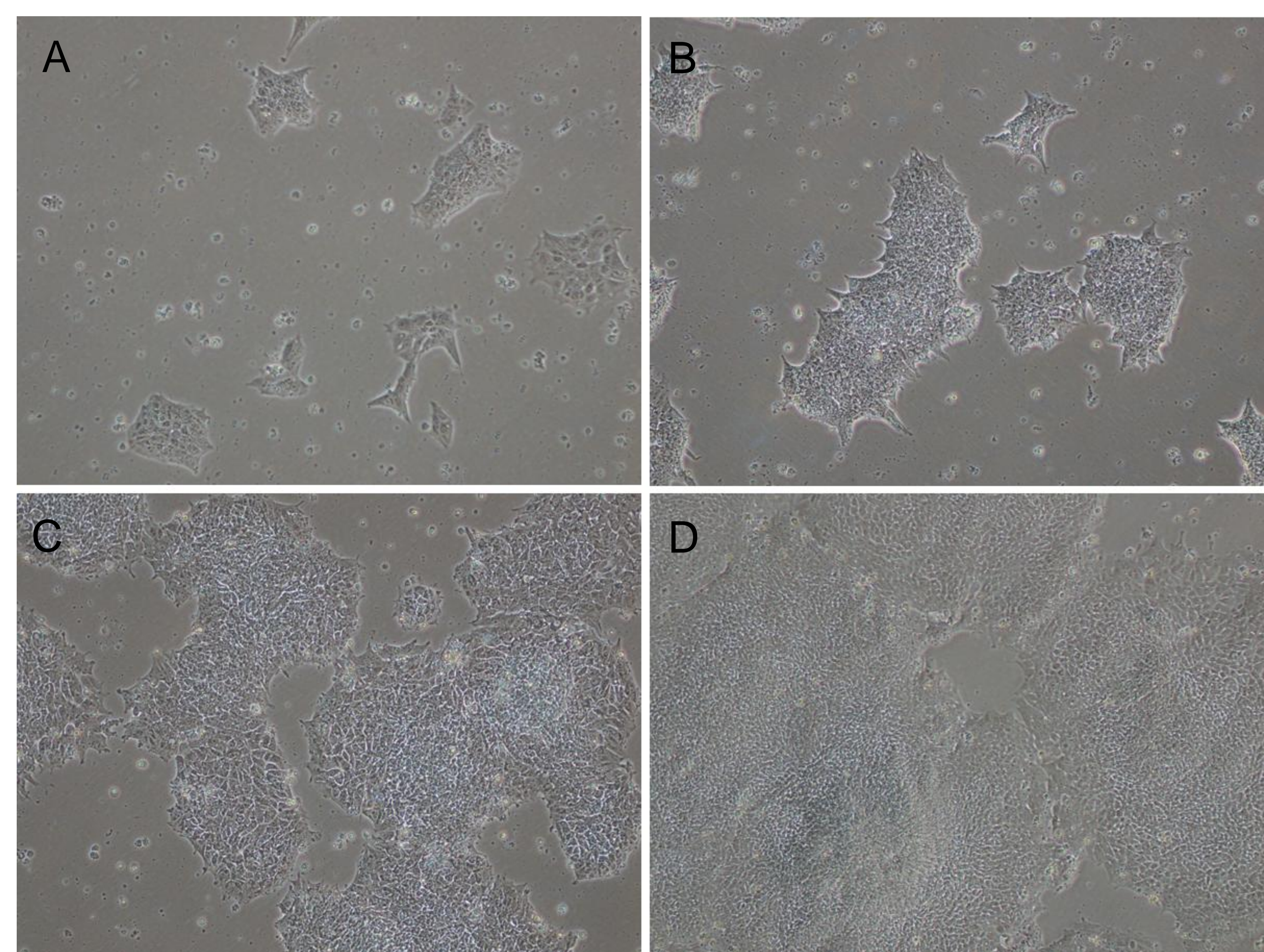


Fig. 2. A: hPSCs cultured in feeder-free condition (Essential 8) at day 1 of splitting (Day 0 of neural induction). **B-D**: The morphology of cells at day 2 (B), 4 (C) and 7(D) after neural induction.

3. The expression of pluripotent and neural markers of induced NSCs

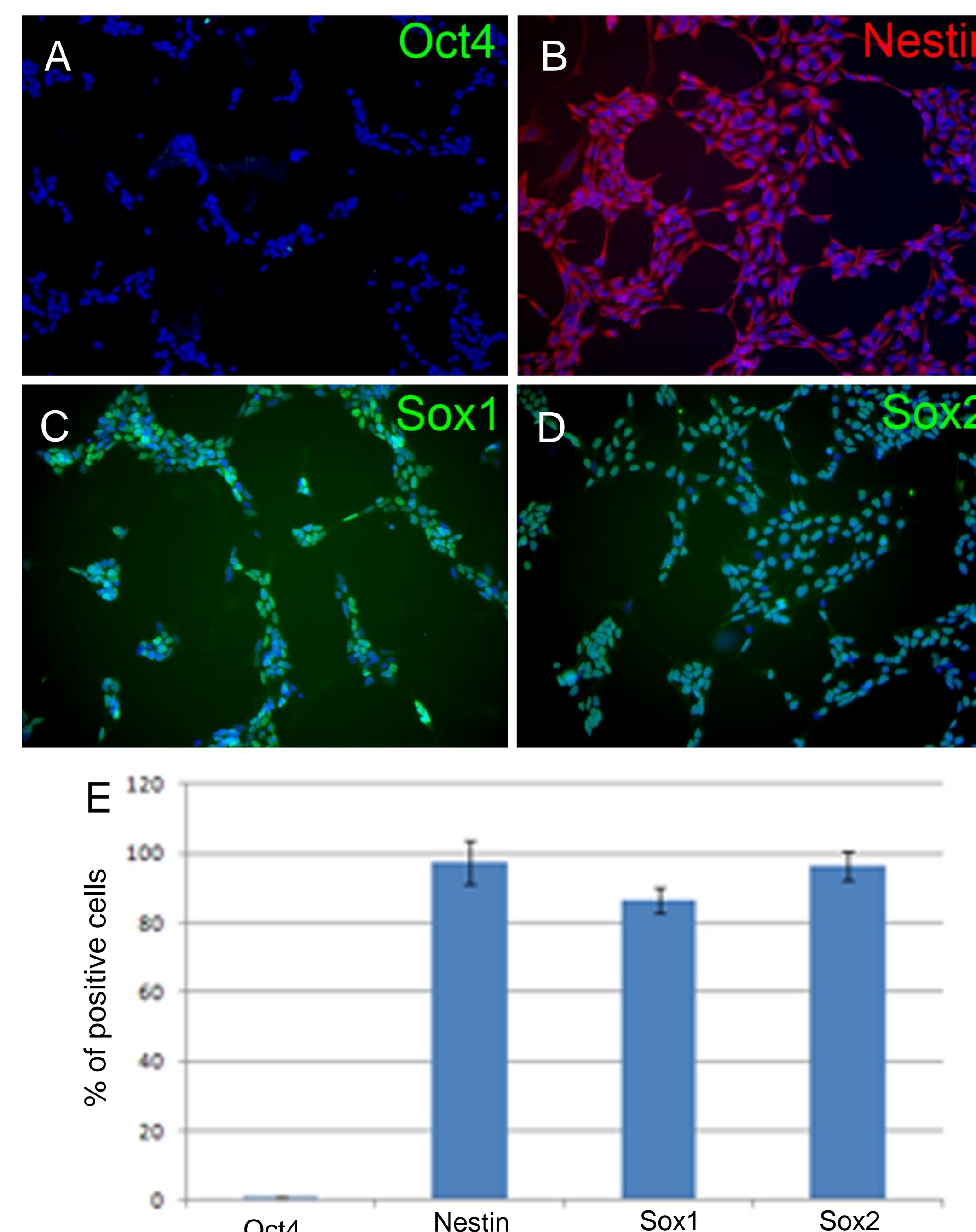


Fig. 3. A-E: NSCs derived from hPSCs were dissociated and plated for staining with antibodies against pluripotent marker Oct4 (**A**), neural marker Nestin (**B**), Sox1 (**C**) and Sox2 (**D**). Cell nuclei were stained with DAPI (blue). **E**: Quantification of positive cells over total cells.

4. NSC expansion

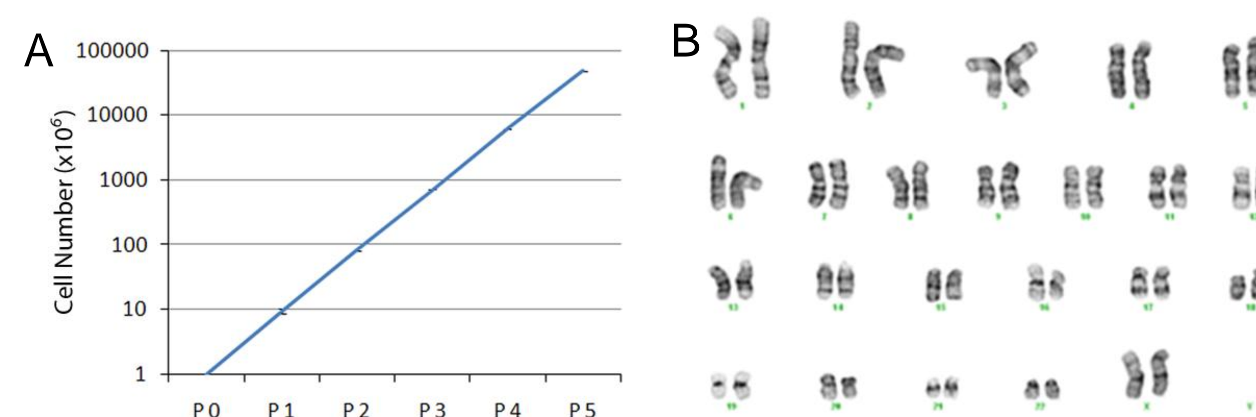


Fig. 4. A: The dynamics of NSC expansion with 8-10 fold increase in cell number of each passage. **B:** No gross chromosomal aberrations were observed by karyotyping of expanded NSCs**.**

5. NSC Differentiation into triple neural lineages

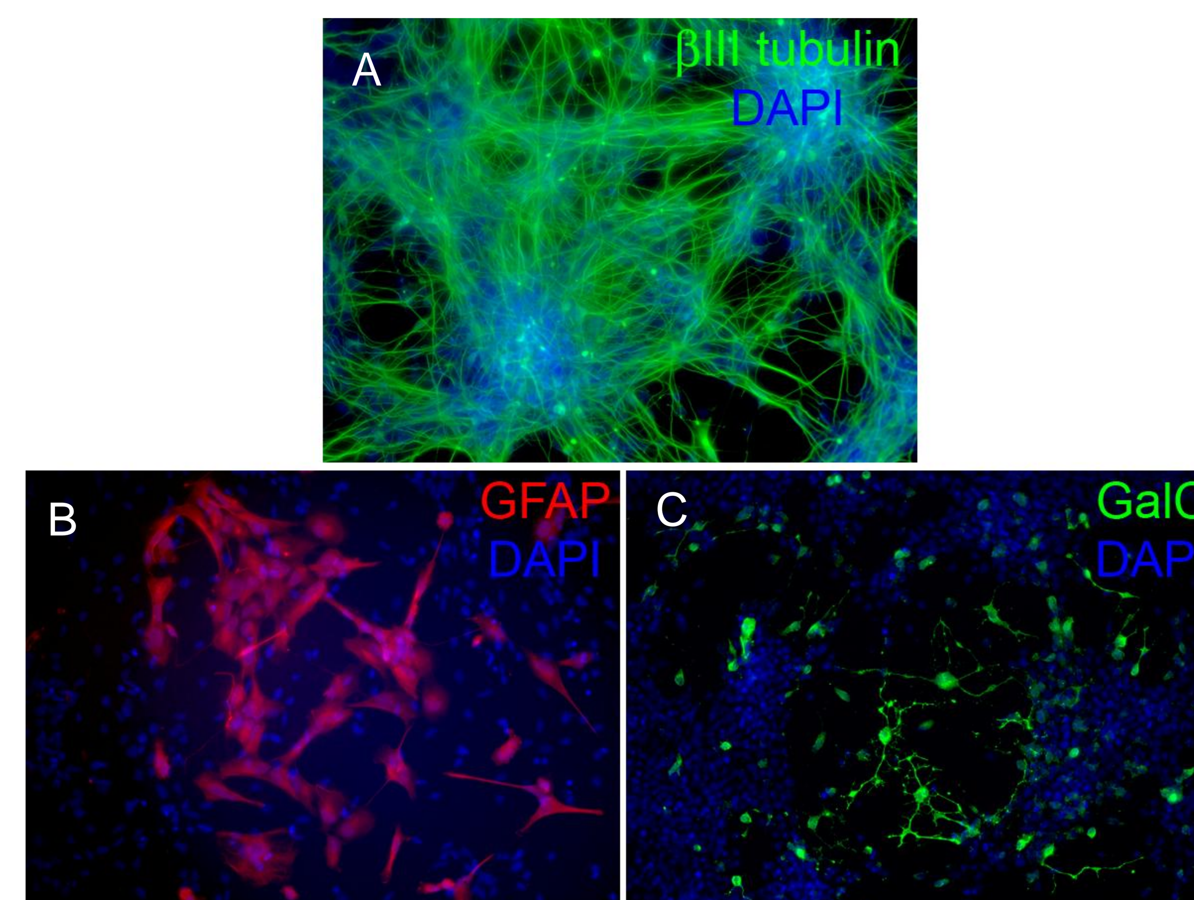


Fig. 5. Differentiated cells from NSCs were stained with antibodies against neuronal maker beta III tubulin (**A**), astrocytic marker glial fibrillary acidic protein (GFAP, **B**) and oligodendrocye marker galactosylceramidase (GalC, **C**). Cell nuclei were stained with DAPI (blue)..

6. Sub-types of neuronal differentiation of expanded hPSC derived NSCs

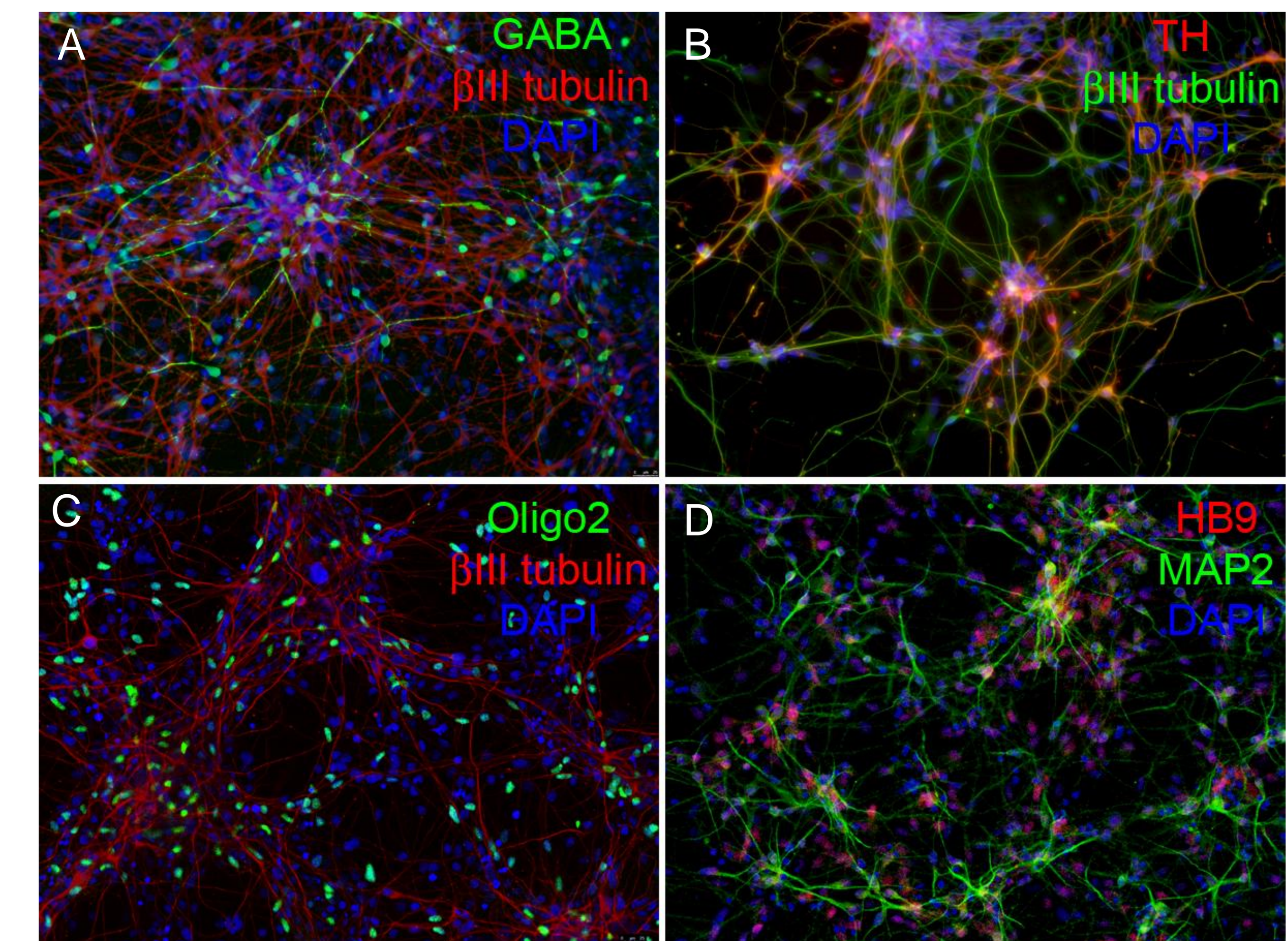


Fig.6. Differentiated cells were stained with antibodies against neuronal marker bIII tubulin (**A, B, C**) or MAP2 (**D**). Regional specific neuronal sub-types were evaluated by staining with antibodies against GABAergic marker GABA (**A**), dopaminergic marker TH (**B**), and motor neuron marker Oligo2 (**C**) and HB9 (**D**). Cell nuclei were stained with DAPI (blue).

7. Comparison of gene expression profiles of primitive NSC to rosette derived NSC and human fetal cortex isolated NSC

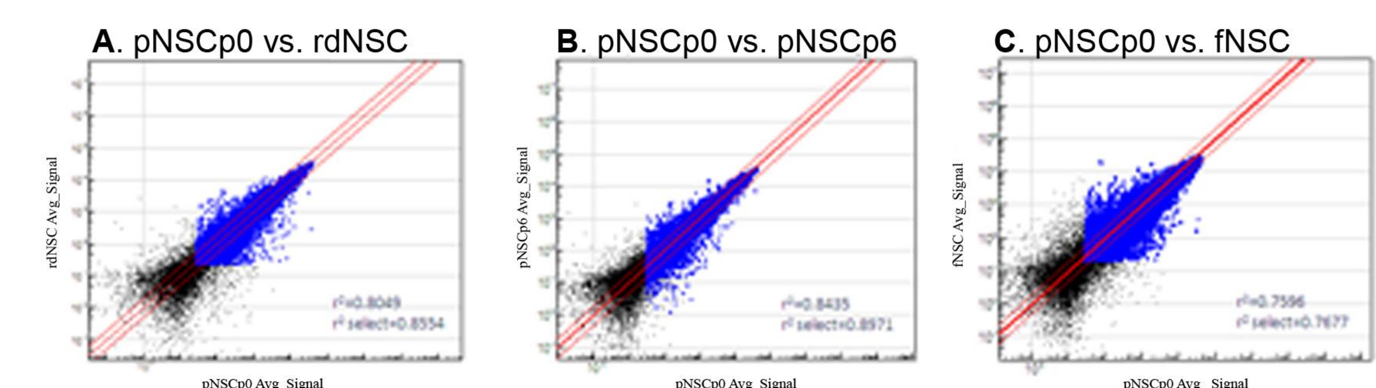


Fig.7. The population similarity was shown in scatter plots. Correlation coefficient of whole genes (r^2) or of expressed genes by both samples (r^2 select) were calculated by Genomestudio software. **pNSCp0**: NSCs at day 7 in neural induction medium. **pNSCp6**: pNSCp0 were passaged for 6 times. **rdNSC**: Rosette derived NSC, > 28 passages (LIFE N7800-100). **fNSC**: Fetal cortex derived NSCs.

SUMMARY

- Neural induction medium induces NSCs from hPSCs in 7 days without EB formation.
- Neural induction medium works for multiple hPSC lines including hESC and hiPSC cultured in both feeder-free and feeder contained conditions.
- Induced NSCs do not express pluripotent marker Oct4 but express neural marker including Nestin, Sox1 and Sox2.
- The efficiency of neural induction is 80-90%.
- Induced NSCs can be cryo-preserved, expanded with 8-10 times increase in cell number of each passage.
- Expanded NSCs have the capacity to be differentiated into neurons, astrocytes and oligodendrocytes.
- Expanded NSCs retain the plasticity for regional specification to subtypes of neurons.
- Global gene expression of the transcripts of NSCs derived by neural induction medium was comparable to rosette derived and human fetal derived NSCs.

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