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Lab Resource: Stem Cell Line

Generation of human embryonic stem cell line expressing green fluorescent protein

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ABSTRACT

Human embryonic stem cell line BJNhem20 pCAG-EGFP was generated using non-viral method (Fig. 1). The construct pCAG-EGFP was transfected using microporation procedure

Resource Table

Name of Stem Cell line	BJNhem20 pCAG-EGFP
Institution	Jawaharlal Nehru Centre for Advanced Scientific Research
Person who created resource	Maneesha S. Inamdar
Contact person and email	inamdar@jncasr.ac.in
Date archived/ stock date	June 5, 2012
Origin	Human embryonic stem cells
Type of resource	Biological reagent: genetically modified human embryonic stem cells
Sub-type	cell line
Key transcription factors	Oct4, Nanog
Authentication	Identity of the cell line confirmed
Link to related literature	http://online.liebertpub.com/doi/abs/10.1089/scd.2008.0131 http://link.springer.com/article/10.1007%2Fs11626-010-9277-3 http://link.springer.com/protocol/10.1007%2F978-1-61779-794-1_9
Information in public databases	—
Ethics	Competent authority approval obtained

Resource details

To generate BJNhem20 pCAG-EGFP cell line, we transfected BJNhem20 by microporation at 1100 V, 30 ms pulse width and 1 pulse number. Expression of pluripotent stem cell markers OCT4, SSEA3, TRA1-60 and TRA1-81 has been shown by immunostaining. Differentiation of BJNhem20 pCAG-EGFP to all the three germ layers was demonstrated by immunostaining for beta III tubulin (ectodermal), Brachyury (mesodermal) and AFP (endodermal). Karyotype was also checked in these cells and found to be normal.

Materials and methods

Cell culture

hESC line BJNhem20 was derived from the grade III embryos and tested for its pluripotency (Inamdar et al., 2009) (Fig. 2). The cell line was included in the analysis of genetic stability in the International Stem Cell Initiative project (Nature Biotech, 2011). This cell line has been cultured continuously for over 200 passages without acquiring karyotypic abnormality (Venu et al., 2010). BJNhem20 cultures were maintained on mouse embryonic fibroblasts (MEFs) feeder layers in hESC medium composed of KnockOut Dulbecco's modified Eagle medium (KO-DMEM—Life Technologies) supplemented with 20% KnockOut Serum Replacement (KOSR—Life Technologies), 1% GlutaMAX (Life Technologies), 1% non-essential amino acids (NEAA—Life Technologies), 0.1% β-mercaptoethanol (Life Technologies) and 8 ng/ml human recombinant basic fibroblast growth factor (human bFGF—Sigma). These cells were routinely passaged by mechanical cutting of undifferentiated colonies.

Microporation of hESCs to generate BJNhem20 pCAG-EGFP cell line

For microporation BJNhem20 cells grown on Matrigel (Becton Dickinson Cat. No. 354277) coated dishes to 60–70% confluency were trypsinized with 1X TrypLE (Life Technologies) at room temperature for 10 min. The cells were pelleted by centrifugation 1000 rpm for 2 min, washed with phosphate buffered saline and counted. 0.4 million cells were mixed with 5 μg of plasmid DNA and suspended in 10 μl of R buffer and the transfection was done at 1100 V, 30 ms and 1 pulse in a microporator according to the manufacturer's instructions (Neon Life Technologies). Cells were then directly seeded onto Matrigel-coated

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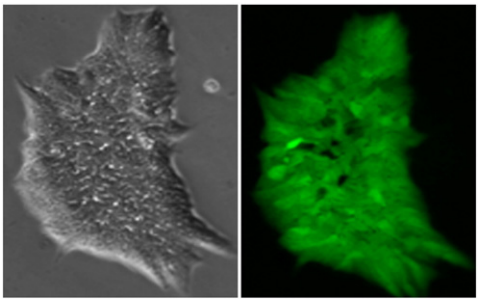

Fig. 1. An isolated colony from BJNhem20 pCAG-EGFP cell line grown on Matrigel imaged in phase (left panel) and fluorescence (right panel) showing GFP expression.

dishes containing mTESR media (Stem Cell Technologies) supplemented with ROCK inhibitor (10 μM Sigma Aldrich) for 24 h. 48 h after transfection puromycin (0.5 μg/ml) was added to the transfected culture and selection continued for 8 days. Puromycin resistant GFP+ cells were subsequently cultured in feeder free conditions on Matrigel in the presence of mTESR medium. After few passages cells were plated sparse and single colonies isolated for clonal culture to obtain GFP expressing BJNhem20 pCAG-EGFP hESC line. Long term culture was maintained on MEFs as described above.

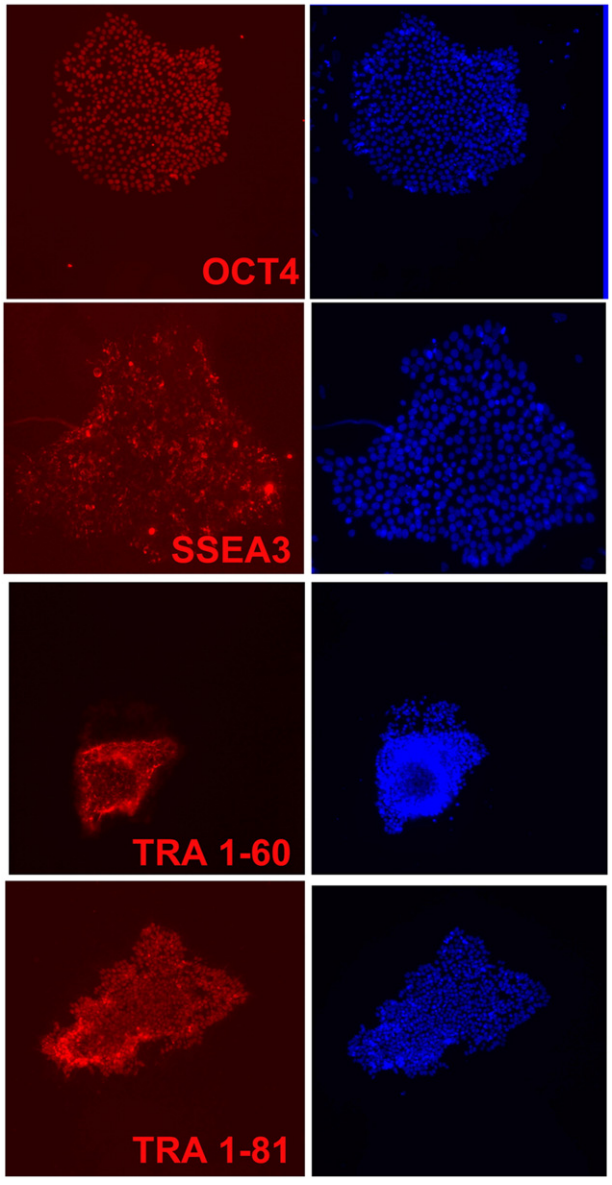

Fig. 2. Pluripotency marker expression in BJNhem20 pCAG-EGFP by immunostaining for Oct4, SSEA3, Tra1-60 and Tra1-81.

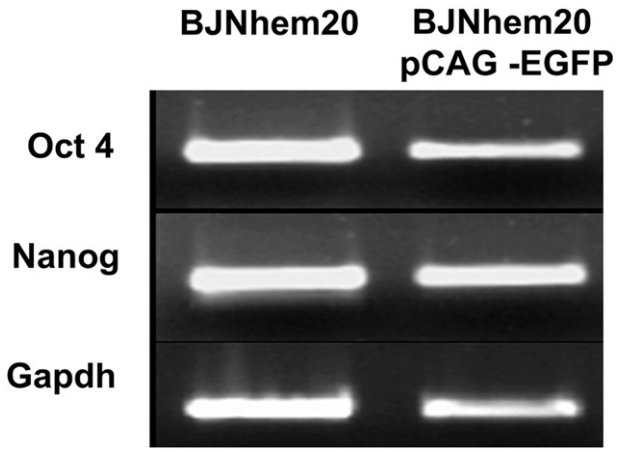

Fig. 3. RT PCR analysis of BJNhem20 pCAG-EGFP

Embryoid body (EB) differentiation

Differentiation of embryoid bodies was carried out as described before (Inamdar et al., 2009). Briefly, colonies grown on MEFs were mechanically cut and pieces cultured in hEB media (10% FBS-Hyclone-SH30070) with β-mercaptoethanol and GlutaMAX in DMEM) on low attachment plates. The hESC aggregates were allowed to grow in suspension for 3 days after which EB like structures were transferred to tissue culture dishes precoated with 0.1% gelatin and allowed to attach and differentiate spontaneously. Embryoid bodies were harvested from day 6 for analyses by immunostaining and also checked for GFP expression.

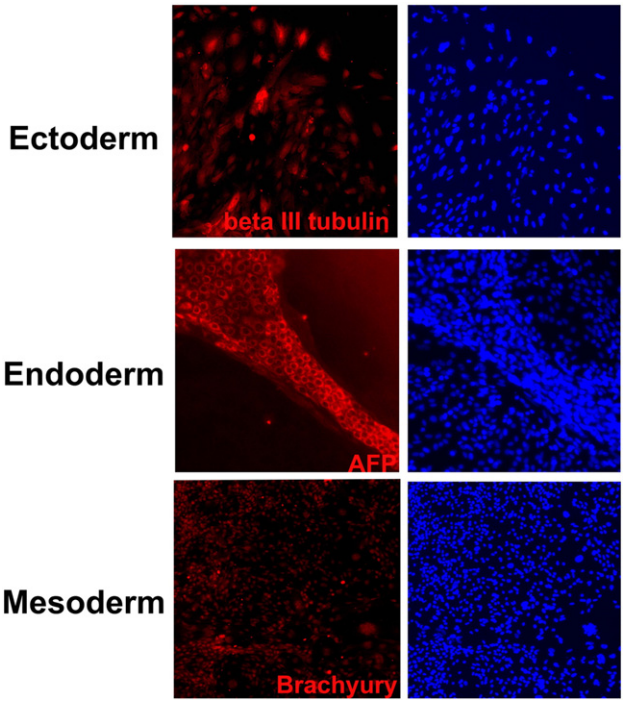

Fig. 4. Differentiation analysis of BJNhem20 pCAG-EGFP: Embryoid bodies stained to show differentiation to all the three germ layers by immunostaining (red) for AFP, Brachyury and β III-Tubulin marking the endoderm, mesoderm and ectoderm respectively.

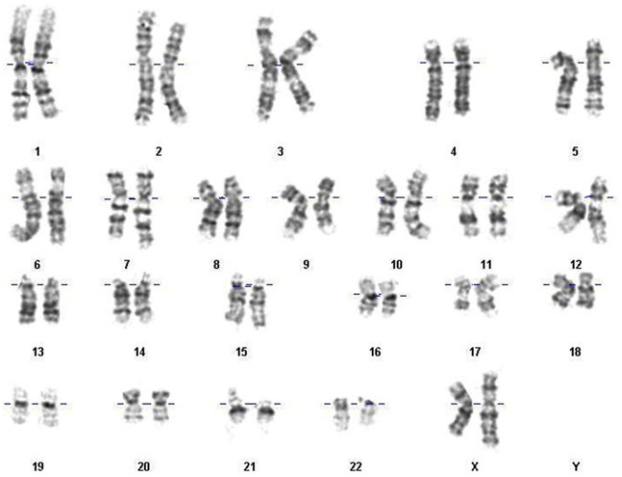

Fig. 5. Karyotype analysis of BJNhem20 pCAG-EGFP.

Immunostaining

hESC and hEBs were fixed in 2% paraformaldehyde in PBS, permeabilized with Triton X (0.1% for cells and 0.3% for EBs) and then blocked with 5% FBS for 1 h at room temperature. Post blocking, cells were incubated with the appropriate primary antibody at 4 °C for 8–12 h, washed with FBS and then incubated with secondary antibody conjugated to Alexa flour 568 (Alexa Flour-1:400) for 1 h, washed and stained with DAPI to visualize nuclei. Images were acquired on an epifluorescence microscope (IX 70 and IX 81 Olympus Corporation) fitted with a cooled CCD camera (CoolSnap, Roper Scientific). Primary antibodies used were against OCT4 (BD Biosciences), TRA1-60 and TRA1-81 monoclonal antibody (kind gift from Peter Andrews, University of Sheffield, UK); SSEA3 (Chemicon, USA); Brachyury (Santa Cruz Biotech); and beta-III tubulin and AFP (Sigma Chemical Co., USA) (Figs. 2 and 4).

RNA isolation/cDNA synthesis and PCR

Total RNA was extracted from cells by TRIzol reagent (Life Technologies Inc., USA) following the manufacturer's instruction. 2 μg of DNase treated RNA was converted to cDNA by performing reverse transcription using Superscript II (Life Technologies Inc., USA). Primer sequences (5′-3′) were as follows: OCT4_F: GAAGGTATTCAGCCAAACGAC; OCT4_R: GTTACAGAACCACACTCGGA; NANOG_F: TGCAAATGTCTTCT GCTGAGAT; NANOG_R: GTTCAGGATGTTGGAGAGTTC; GAPDH_F: GTCCATGCCATCACTGCCA; GAPDH_R: TTACTCCTTGGAGCCATG (Fig. 3).

Verification of karyotype

Standard G banding of metaphase spreads was performed and analyzed using CytoVision software. For each sample, at least 50 metaphases were counted and 10 were analysed (Fig. 5).

Acknowledgments

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References

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