Lab Resource: Stem Cell Line

Generation of human embryonic stem cell line expressing green fluorescent protein

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A R T I C L E   I N F O

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A B S T R A C T

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 © 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Resource Table

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</tr>
<tr>
<td>Person who created resource</td>
<td>Maneesha S. Inamdar</td>
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<tr>
<td>Contact person and email</td>
<td><a href="mailto:inamdar@jncasr.ac.in">inamdar@jncasr.ac.in</a></td>
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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% confluence 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 microrapator according to the manufacturer’s instructions (Neon Life Technologies). Cells were then directly seeded onto Matrigel-coated...
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.

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. 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.

**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

**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.
**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 Fluor-1:400) for 1 h, washed and stained with DAPI to visualize nuclei. Images were acquired on an epi-fluorescence 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: GAAGGTATTCAGCAAACGC; OCT4_R: GTTACGAACCCACTCGGA; NANOG_F: TGCAATGTCCCTCTGCTGAGAT; NANOG_R: GTTCAGAGTGTGGAGGATGTC; GAPDH_F: GTCCATGCCACACTGCA; GAPDH_R: TACTCTGGAGGCATG (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**

