Stem Cell Research 16 (2016) 338–341

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Stem Cell Line

Generation of human control iPS cell line *CHOPWT10* from healthy adult peripheral blood mononuclear cells

Jean Ann Maguire [a,b], Alyssa L. Gagne [a,b], Chintan D. Jobaliya [a,b], Shilpa Gandre-Babbe [c], Paul Gadue [a,b], Deborah L. French [a,b,*]

[a] Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia, United States
[b] Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, University of Pennsylvania, United States
[c] Coriell Institute for Medical Research, United States

ARTICLE INFO

Article history:
Received 29 January 2016
Accepted 1 February 2016
Available online 3 February 2016

ABSTRACT

The *CHOPWT10* iPS cell line was generated to be used as a control for applications such as in differentiation analyses to the three germ layers and derivative tissues. Peripheral blood mononuclear cells (PBMCs) obtained from a healthy adult male were reprogrammed using the non-integrating Sendai virus expressing Oct3/4, Sox2, c-Myc, and Klf4.

Resource table: *CHOPWT10*

Name of stem cell construct	*CHOPWT10*
Institution	The Children's Hospital of Philadelphia (CHOP)
Person who created resource	Jean Ann Maguire
Contact person and email	Deborah L. French, frenchd@email.chop.edu
Date archived/stock date	2014
Origin	Human PBMCs
Type of resource	Biological reagent: iPS cell from human PBMCs
Subtype	Cell line
Key transcription factors	Oct4, Sox2, Klf4, c-Myc
Authentication	Identity and purity of cell line confirmed (Figs. 1 and 2)
Link to related literature	N/A
Information in public databases	N/A

Resource details

To generate *CHOPWT10* cells, four Yamanaka reprogramming factors (Oct3/4, Sox2, c-Myc, and Klf4) were delivered into mononuclear cells through Sendai viral infection. The Sendai virus is non-integrating and the absence of the vectors in the reprogrammed cells was confirmed by PCR analysis (Fig. 1A).

DNA fingerprinting confirmed the genetic relation of the derivative line to the parental somatic line (Fig. 1B). The cells exhibited normal karyotype (46, XY) upon G-band analysis (Fig. 1C).

Pluripotency was verified by gene expression of pluripotent stem cell markers Oct4, Sox2, Nanog, Rex1, DMNT3B and ABCG2 by qPCR (Fig. 1D). In addition, pluripotent surface marker expression at single cell resolution of SSEA3, SSEA4, Tra-1-60, and Tra-1-81 were confirmed by flow cytometry (Fig. 1E). Differentiation capacity into three germ layers was confirmed by PluriTest™ and quantitative PCR (Fig. 2).

Materials and methods

Blood sample collection and processing

Blood samples were collected from a healthy adult male into BD Vacutainer cell preparation tubes. The PBMCs were isolated by centrifugation at 1800 rcf for 30 min, after which mononuclear cells were transferred to a fresh tube, washed with PBS, and pelleted at 300 rcf for 10 min. Pellets were resuspended in freezing media (90% FBS, 10% DMSO) and stored in liquid nitrogen. Blood collection was approved by the CHOP Institutional Review Board committee with written informed consent.

iPS cell reprogramming

The PBMCs were expanded and reprogrammed as previously described (Yang et al., 2012). Briefly, PBMCs were expanded in QBSF-60 medium containing EPO (2 U/mL), IGF-1 (40 ng/mL), SCF (50 ng/mL), IL-3 (10 ng/mL), dexamethasone (1.5 μM), ascorbic

* Corresponding author at: 3501 Civic Center Blvd, CTRB 5014, Philadelphia, PA 19104, United States.

http://dx.doi.org/10.1016/j.scr.2016.02.017

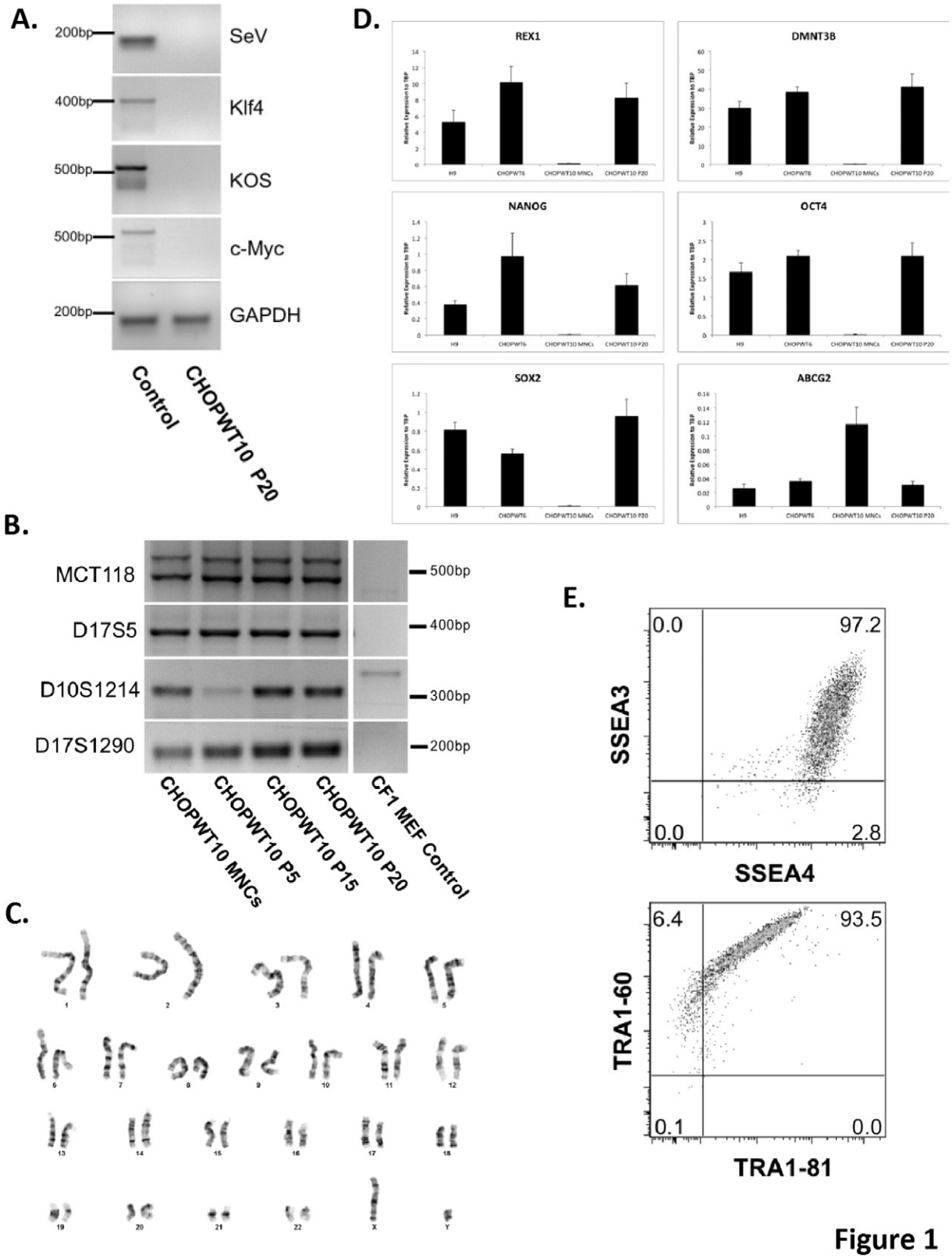

Figure 1

Fig. 1. *Generation and characterization of CHOPWT10.* (A) PCR analysis of reprogramming genes after 20 passages of *CHOPWT10* using GAPDH as a loading control. Cells harvested immediately post-Sendai infection were used as a positive control. (B) *CHOPWT10* iPS cells compared to parental cells by DNA fingerprinting. (C) Karyotype analysis of *CHOPWT10*. (D) Quantitative PCR of relative gene expression of pluripotency genes compared to embryonic stem cells (H9) and a control iPS cell line (CHOPWT6). (E) Flow cytometry profiles of surface markers on undifferentiated cells: SSEA3, SSEA4, TRA-1-60, and TRA-1-81.

acid (50 ng/mL), glutamine (1%), and penicillin/streptomycin (1%) for 7 days. Cells were transduced with Sendai virus expressing human Oct3/4, Sox2, Klf4, and c-Myc. (Life Technologies) (moi according to manufacturer's instructions). Transduced cells were plated on culture dishes containing irradiated mouse embryonic fibroblasts (MEFs), and maintained in human embryonic stem