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Lab resource: Stem cell line

Generation of a human iPSC line from a patient with a defect of intergenomic communication

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ABSTRACT

Human iPSC line PG64SV.2 was generated from fibroblasts of a patient with a defect of intergenomic communication. This patient harbored a homozygous mutation (c.2243G>C; p.Trp748Ser) in the gene encoding the catalytic subunit of the mitochondrial DNA polymerase gamma gene (*POLG*). Reprogramming factors Oct3/4, Sox2, Klf4, and cMyc were delivered using a non integrative methodology that involves the use of Sendai virus.

1. Resource table

Name of stem cell line	PG64SV.2
Institution	Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols", Facultad de Medicina, (UAM-CSIC) and Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER) Madrid, Spain. Instituto de Investigación Hospital 12 de Octubre ("i + 12"), Madrid, Spain.
Person who created resource	Francisco Zurita
Contact person and email	M. Esther Gallardo, egallardo@iib.uam.es
Date archived/stock date	July 20, 2014
Origin	Human skin cells
Type of resource	Biological reagent: induced pluripotent stem cells (iPSC) from a patient with a defect of intergenomic communication
Sub-type	Cell line
Key transcription factors	Oct3/4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	None
Information in public databases	http://www.eurobiobank.org/en/services/services.htm

2. Resource details

The generation of the human iPSC line, PG64SV.2, was carried out using non-integrative Sendai viruses containing the reprogramming factors, Oct3/4, Sox2, cMyc, Klf4 (Takahashi et al., 2007). For this purpose, fibroblasts from a patient with a defect of intergenomic communication (Hirano et al., 2001) were provided by EuroBiobank. The patient's fibroblasts carried a homozygous mutation in the gene encoding the catalytic subunit of the mitochondrial DNA polymerase gamma (*POLG*) (c.2243G>C; p.Trp748Ser). The presence of this mutation in the iPSC line was confirmed by Sanger sequencing (Fig. 1A). PG64SV.2 iPSC colonies displayed a typical ES-like colony morphology and growth behavior (Fig. 1B) and they stained positive for alkaline phosphatase activity (Fig. 1C). We confirmed the clearance of the vectors and the exogenous reprogramming factor genes by RT-PCR after eight culture passages (Fig. 1D). The endogenous expression of the pluripotency associated transcription factors Oct4, Sox2, Klf4, Nanog, Crypto and Rex1 was also evaluated by RT-PCR (Fig. 1E). Immunofluorescence analysis revealed expression of transcription factors *OCT4*, *NANOG*, *SOX2* and surface markers *SSEA3*, *SSEA4*, *TRA1-60* and *TRA1-81* characteristics of pluripotent ES cells (Fig. 1F). Promoters of the pluripotency associated genes, *OCT4* and *NANOG*, heavily methylated in the original fibroblasts were almost demethylated in the PG64SV.2 line suggesting an epigenetic reprogramming to pluripotency (Fig. 1G). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XX) after more than twenty culture passages (Fig. 1H). We also confirmed by DNA fingerprinting analysis that the line PG64SV.2

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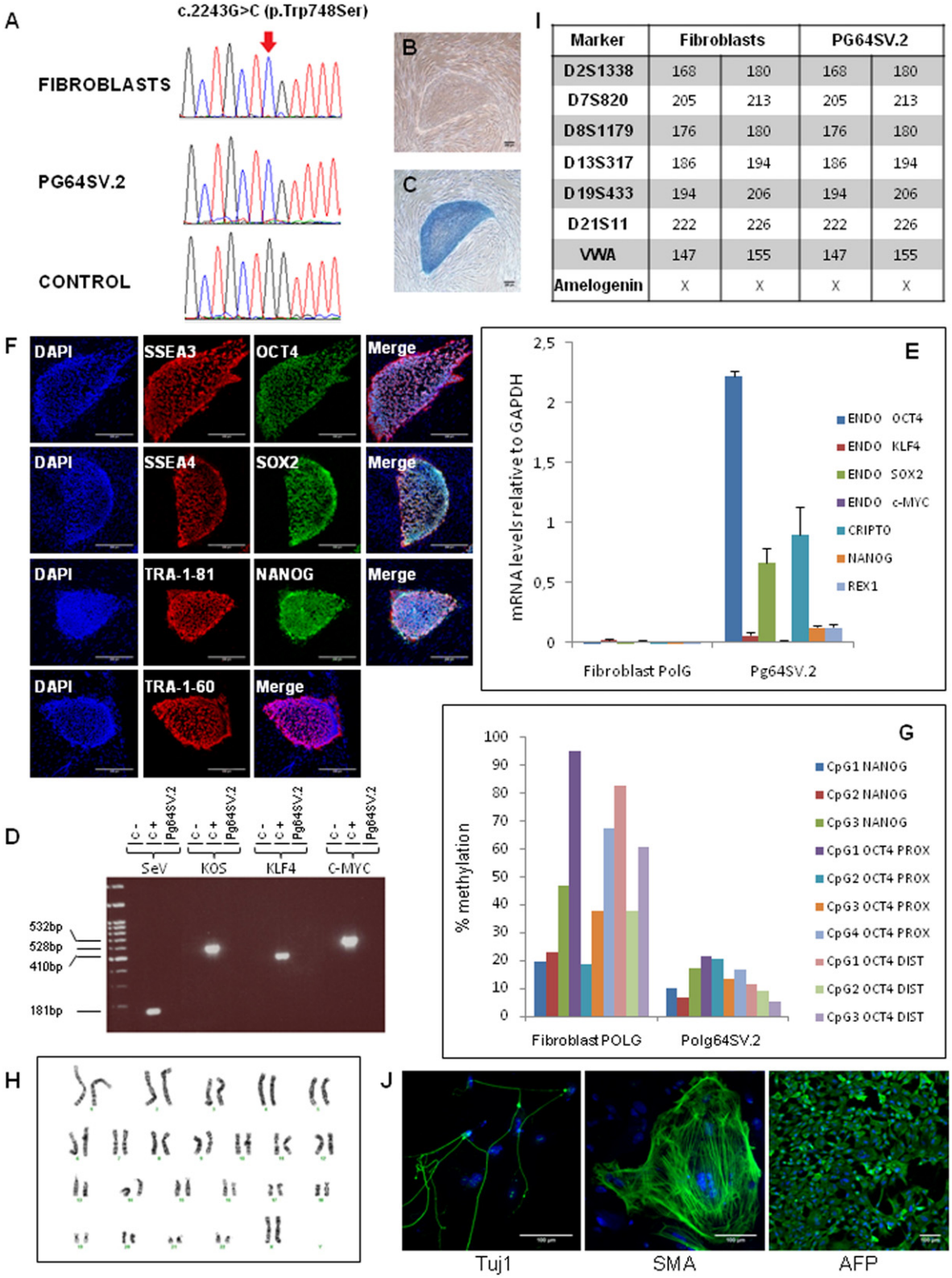

Fig. 1. Molecular and functional characterization of the PG64SV.2 iPSC line. 1A. Electropherograms showing the c.2243G > C mutation in the patient's fibroblasts and in the PG64SV.2 line. 1B. Typical ES-like colony morphology of the PG64SV.2 iPSC line. 1C. Positive phosphatase alkaline staining. 1D. Confirmation by RT-PCR of the exogenous reprogramming factors and sendai virus vector silencing. 1E. QPCR showing the expression of the pluripotency associated markers *NANOG*, *OCT4*, *SOX2*, *KLF4*, *CRIPTO* and *REX1*. 1F. Immunofluorescence analysis showing expression of typical pluripotent ES cell markers such as the transcription factors Oct4, Nanog, Sox2 and the surface markers SSEA3, SSEA4, Tra1-60 and Tra1-81; scale bars: 300 μm. 1G. Bisulfite pyrosequencing of the *OCT4* and *NANOG* promoters. The promoters of the transcription factors, OCT4 and NANOG were almost demethylated in the generated iPSC line. 1H. Karyotype analysis. PG64SV.2 has a normal karyotype (46, XX). 1I. DNA fingerprinting analysis showing that PG64SV.2 comes from the patient's fibroblasts. 1J. Embryoid body based *in vitro* differentiation assays. PG64SV.2 differentiates into all three germ layers, demonstrated by positive AFP endoderm staining (l), positive Tuj1 ectoderm staining and positive SMA mesoderm staining.