Stem Cell Research 16 (2016) 766–769

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Stem Cell Line

Generation of a human iPSC line from a patient with Leigh syndrome caused by a mutation in the *MT-ATP6* gene

Teresa Galera-Monge [a,b,c,d], Francisco Zurita-Díaz [a,b,c,d], Cristina González-Páramos [a,b,d], Ana Moreno-Izquierdo [d,e], Mario F. Fraga [f], Agustin F. Fernández [g], Rafael Garesse [a,b,c,d], M. Esther Gallardo [a,b,c,d,*]

[a] Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain
[b] Instituto de Investigaciones Biomédicas "Alberto Sols", (UAM-CSIC) Madrid, Spain
[c] Centro de Investigación Biomédica en Red (CIBERER), Madrid, Spain
[d] Instituto de Investigación Hospital 12 de Octubre ("i + 12"), Madrid, Spain
[e] Servicio de Genética, Hospital 12 de Octubre, Madrid, Spain
[f] Nanomaterials and Nanotechnology Research Center (CINN-CSIC), Spain
[g] Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA), HUCA, Universidad de Oviedo, Oviedo, Spain

ARTICLE INFO

Article history:
Received 12 April 2016
Accepted 13 April 2016
Available online 18 April 2016

ABSTRACT

Human iPSC line L749.1 was generated from fibroblasts of a patient with Leigh syndrome associated with a heteroplasmic mutation in the *MT-ATP6* gene. Reprogramming factors OCT4, SOX2, CMYC and KLF4 were delivered using retroviruses.

1. Resource table

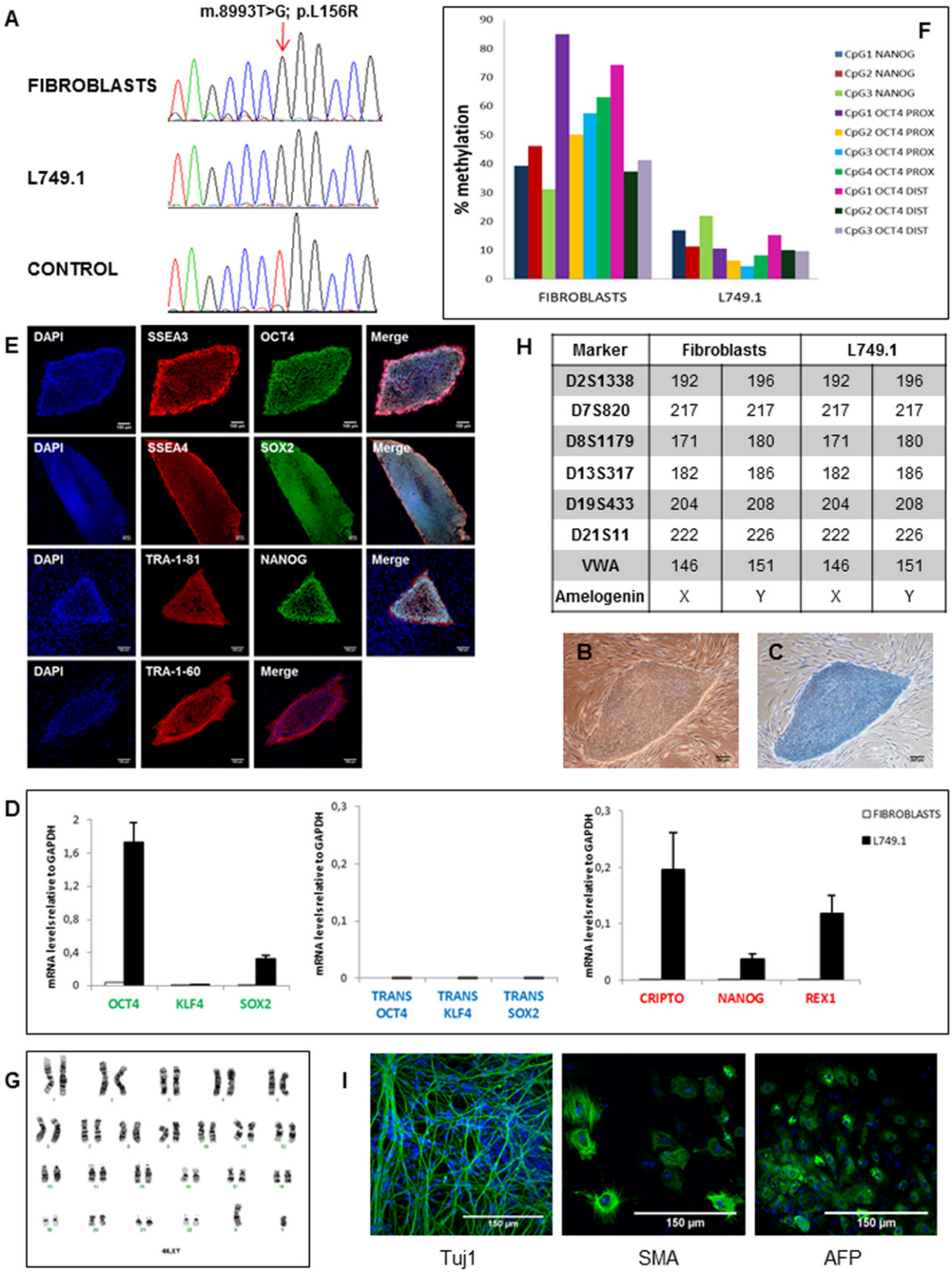
Name of stem cell line	L749.1
Institution	Departamento de Bioquímica, Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Facultad de Medicina, Universidad Autónoma de Madrid and Centro de Investigación Biomédica en Red en Enfermedades Raras (CIBERER) Madrid, Spain. Instituto de Investigación Hospital 12 de Octubre ("i+12"), Madrid, Spain.
Person who created resource	Teresa Galera-Monge
Contact person and email	M. Esther Gallardo, egallardo@iib.uam.es
Date archived/stock date	April 2013
Origin	Human skin cells
Type of resource	Biological reagent: Induced pluripotent stem cells (iPSC) from a patient with Leigh syndrome due to a mutation in the *MT-ATP6* gene
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, CMYC, KLF4
Authentication	Identity and purity of cell line confirmed (Fig 1)
Link to related literature	None
Information in public databases	None

2. Resource details

The generation of the human iPSC line, L749.1, was carried out using retroviruses harboring the reprogramming factors, *OCT4*, *SOX2*, *CMYC*, *KLF4* (Takahashi et al., 2007). For this purpose, fibroblasts from a described patient presenting with Leigh syndrome and hypertrophic cardiomyopathy were obtained. The patient's fibroblasts carried a heteroplasmic mutation (90%) in the *MT-ATP6* gene (c.8993T>G; p. Leu156Arg) (Pastores et al., 1994). The presence of this mutation in the iPSC line was evaluated and confirmed by Sanger sequencing (Fig. 1A). L749.1 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 silencing of the retroviral transgenes by quantitative RT-PCR using primers specific for either the endogenous or transgenic factors *OCT4*, *SOX2* and *KLF4* (Fig. 1D). The pluripotency associated transcription factors *NANOG*, *CRIPTO* and *REX1* were also evaluated by RT-PCR (Fig. 1D). 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. 1E). Promoters of the pluripotency associated genes, *OCT4* and *NANOG*, heavily methylated in the original fibroblasts were almost demethylated in the L749.1 line suggesting an epigenetic reprogramming to pluripotency (Fig. 1F). The iPSC line has been adapted to feeder-free culture conditions and displays a normal karyotype (46, XY) after more than twenty culture passages (Fig. 1G). We also confirmed by DNA fingerprinting analysis that the line L749.1 was derived from the patient's fibroblasts (Fig. 1H). Finally, the capacity of the generated iPSC line to differentiate into the

* Corresponding author at: Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Madrid, Instituto de Investigaciones Biomédicas "Alberto Sols" (UAM-CSIC), Avda Arzobispo Morcillo s/n 28029, Madrid, Spain.
E-mail addresses: egallardo@iib.uam.es, esther.gallardo@ciberer.es (M.E. Gallardo).

http://dx.doi.org/10.1016/j.scr.2016.04.012



Marker	Fibroblasts		L749.1	
D2S1338	192	196	192	196
D7S820	217	217	217	217
D8S1179	171	180	171	180
D13S317	182	186	182	186
D19S433	204	208	204	208
D21S11	222	226	222	226
VWA	146	151	146	151
Amelogenin	X	Y	X	Y

Fig. 1. Molecular and functional characterization of the L749.1 iPSC line. A. Electropherograms showing the mutation in the patient's fibroblasts and in the L749.1 line. **B.** Typical ES-like colony morphology of the L749.1 iPSC line. **C.** Positive phosphatase alkaline staining. **D.** QPCR showing the expression of the pluripotency associated markers *NANOG*, *OCT4*, *SOX2*, *KLF4*, *CRIPTO* and *REX1*. **E.** 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: 300µm. **F.** 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. **G.** Karyotype analysis. L749.1 has a normal karyotype (46, XY). **H.** DNA fingerprinting analysis showing that L749.1 comes from the patient's fibroblasts. **I.** Embryoid body based *in vitro* differentiation assays. L749.1 differentiates into all three germ layers, demonstrated by positive AFP endoderm staining, positive Tuj1 ectoderm staining and positive SMA mesoderm staining.