BANCO NACIONAL DE LÍNEAS CELULARES (TRONCALES)

National Bank of Stem Cell Lines

IMPRESO DE SOLICITUD DE DEPÓSITO DE UNA LÍNEA

Application Form to Deposit a Human Cell Line

Documentos que se acompañan:
Attached documents:

☐ Copia de la autorización de derivación de la línea celular, junto con informe del Comité Ético del centro de procedencia.
A copy of the authorization for the derivation of the cell line, with the corresponding ethics committee approval

☐ Copia de cualquier publicación científica relacionada con la derivación y/o caracterización de la línea.
A copy of any relevant published scientific papers related to the derivation and/or characterization of the cell line

☐ C. V. del investigador principal (una página; formato libre).
A one page CV for the Principal Investigator

☐ Otros (especificar).
Others (specify)

SECCIÓN 1 — Información General
Section 1 — General Information

Nombre de la línea: hiPSC clone 4
Name of the line:

Investigador principal: Majlinda Lako and Lyle Armstrong
Principal Investigator:

Origen de la línea celular: Adult human skin fibroblasts (AHDF)
Origin of the cell line

Embrionario ☐ *Embryonic* **Fetal** ☐ *Fetal* **Adulto** ☒ *Adult*

¿La línea celular ha sido derivada de un embrión con anomalía genética?
Has the cell line been derived from an embryo with genetic anomaly?

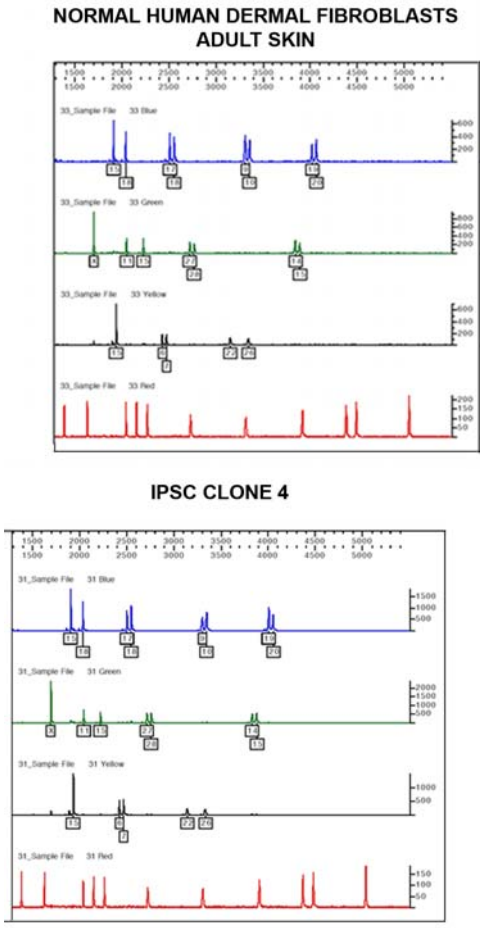
NO ☒ *No* **SÍ** ☐ *Yes* (especificar) *(specify)*

Identificación genética de la línea celular. Método y resultado
Genetic identity of the cell line. Method and result

Method: DNA fingerprinting

To confirm that hiPSC clone 4 was of identical origin to adult fibroblasts, we carried out DNA fingerprinting. Total genomic DNA was extracted from all three samples and amplified with 11 microsatellite markers: D3S1358, vWA, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, TH01, and FGA and analysed on an ABI 377 sequence detector using Genotype software (Applied Biosystems, Foster City, CA; http://www.appliedbiosystems.com).

Result: DNA fingerprinting of hiPSC clone 1, 4 and AHDF showing identical DNA genetic profile.



SECCIÓN 2
Section 2

Datos del Depositante
Applicant Details

Investigador Principal: *Principal Investigator:* Majlinda Lako and Lyle Armstrong	**Dirección Postal:** *Postal address:* **Centro de Investigación Príncipe Felipe** **Avda. Autopista del Saler, 16-3 (junto Oceanográfico)** **46012 VALENCIA (Spain)**
Centro de Trabajo: *Institution:* **Centro de Investigación Príncipe Felipe**	**Teléfono** *(phone)*: **: +34 963289681 Ext. 1211/1204** **Fax: : +34 963289701** **E-mail:** mlako@cipf.es; larmstrong@cipf.es

SECCIÓN 3
Section 3

Datos de la Línea Celular
Details of Cell Line

Tipo de muestra biológica (especificar estadio embrionario, semanas de gestación,...)
Kind of biological sample (specify embryonic stage, weeks of pregnancy,...)

Human induced pluripotent Stem cells

Muestra biológica
Biological simple: adult human skin fibroblasts

Fresco ☐ *Fresh* **Crioconservado** ☒ *Cryopreserved*

Fecha de la obtención del muestra biológica *Date of obtaining the biological sample* July 2008	**Fecha del uso o descongelación** *(si congelado)* *Date used or thawed (if frozen)* Juy 2008
Fecha de la donación del muestra biológica *Date of donation of the biological sample*	

Descripción general del procesamiento previo del muestra biológica utilizado (cultivo embrionario, procesamiento muestra fetal o de tejido adulto)
General description of the processing of the biological sample used (embryonic culture, processing of fetal sample or of adult tissue)

Adult human dermal fibroblasts in the log phase of growth (Lonza) were transduced with retroviral particles. Briefly, the following plasmids: pMXs-hNANOG, pMXs-hOCT4, pMXs-hSOX2, pMXs-hKLF4 and pMXs-hcMYC (Addgene) were packaged into retroviral particles by transfection into Phoenix Amphotropic cells using the Calcium Phosphate transfection kit (Sigma Aldrich, Poole, Dorset, UK http://www.sigmaaldrich.com/). Retroviral transductions were performed twice at a 24 hour interval. Forty eight hours after the first transduction, the adult dermal fibroblasts were disaggregated to single cells by trypsinisation (0.05% Trypsin, Invitrogen) then plated onto feeder layers of mitotically inactivated mouse embryonic fibroblasts in hESC culture medium at a density of 8,000 cells per one well of a six well plate. The feeder plates with retrovirus treated cells were maintained at 37oC / 5% CO2 for 21 days or until colonies of cells with a morphology similar to ESC appeared. These were mechanically dissected into several pieces and plated onto fresh feeder cells to develop further colonies for characterisation. We were able to obtain 7 hiPSC clones from 50,000 transduced adult dermal fibroblasts which results in 0.014% efficiency of reprogramming. Two of the hiPSC clones, named as hiPSC clone 1 and 4 were characterised further and used for all analyses described in this study.

En caso de muestra embrionaria, indicar si se utilizaron blastómeros o células de la masa celular interna y el método de aislamiento utilizado
If of embryonic origin, indicate whether blastomeres or internal cell mass were used, as well as the isolation method
Not applicable

Origen del soporte celular o acelular utilizado para la derivación, así como de los componentes de los medios de cultivo (si se describen en publicación, indicar además referencia)
Origin of the cellular or cellular free support used in derivation in addition to the components of the culture mediums (if they are described in a publication, please indicate the reference).

Armstrong et al. Stem Cells (in press) and attached to this application

Mantenimiento de la línea: *Line maintenance*
Ratio de pase: *Passage ratio 1 to 4*
Método de pase: *Passage method collagenase IV on mitotically inactivated mouse embryonic fibroblasts with human ESC medium*

	si	**no**
Xenobióticos *Xenobiotics*	***Yes***	*No*

Descripción de las características morfológicas de la línea en cultivo (forma y tamaño colonias; forma y tamaño células; ratio núcleo/citoplasma; otros)

Description of the morphological characteristics of the line in culture (form and size of the colonies; form and size of the cells; nucleus/cytoplasm ratio; others)

High nucleus to cytoplasm ratio, identical to human ESC morphology

Controles microbiológicos realizados (indicar detalladamente)

Microbiological controls carried out (indicate in detail) mycoplasma test carried out using Elisa and PCR: result negative

Marcadores:
Markers

	Método (ARN/proteínas) *Method (RNA/proteins)*	**nº pase** *Passage n.*	**resultado** *results*	**comentarios** *comments*
Oct 4	**RNA/protein**	**20, 30**	**pos**	
Nano	**RNA/protein**	**20, 30**	**pos**	
Rex 14	**RNA**	**20, 30**	**pos**	
Sox 24	**RNA/protein**	**20, 30**	**pos**	
SSEA34	**Protein**	**20, 30**	**pos**	
SSEA4	**Protein**	**20, 30**	**pos**	
TRA-1-60	**Protein**	**20, 30**	**pos**	
TRA-1-81	**Protein**	**20, 30**	**pos**	
Telomerasa/*Telomerase*	**RNA**	**20, 30**	**pos**	
Fosfatasa Alk. */Alkaline phosphatase enzyme activity*		**20, 30**	**pos**	
Cariotipo / *Karyotype*	*yes*	**NORMAL**		
Otros / *Others*	*CGH*	*report attached*		

Capacidad de diferenciación
Differentiation capacity

	Ectodermo/ *Ectoderm*			**Endodermo/***Endoderm*			**Mesodermo/** *Mesoderm*		
	marcador *marker*	**pase** *passage*	**resultado** *result*	**marcador** *marker*	**pase** *passage*	**resultado** *result*	**marcador** *marker*	**pase** *passage*	**resultado** *result*
In Vitro *In vitro*	*beta 3 tubulin*	*20-30*	*positive*	*AFP*	*20-30*	*positive*	*CD31*	*20-30*	*positive*

In vivo/ *in vivo* *teratoma* **Método:** *Method:* **SC injections into SCID mice** **Resultado:see next section** *Result:*

Descripción de las características de diferenciación *in vitro*
Description of the differentiation characteristics in vitro

To investigate whether continuous expression of some of the exogenous factors can affect the differentiation potential, both hiPSC clones were removed from the feeder layers and placed either in suspension culture to make EBs or attached to gelatin coated plates in differentiation media as described in Materials and Methods (Figure 3A). Quantitative RT-PCR analysis suggested that *OCT4* expression was significantly downregulated during the 30 day EB differentiation time course similarly to hESC (Figure 3B). Similarly the expression of mesodermal marker, *KDR* (Figure 3B), endodermal marker *AFP* (data not shown) and ectodermal marker, *NESTIN* was upregulated during the differentiation time course in a similar pattern to differentiating hESC. Similar results were obtained by immunohistochemistry (Figure 3C). We also tested the *in vitro* differentiation potential of hiPSC clones using monolayer differentiation conditions as described in the Materials and Methods section. Although there are differences in upregulation of various markers during the 4 week time course, it is clear that both hiPSC clones can give rise to differentiated cells expressing markers of endoderm (as shown by *AFP* expression), mesoderm (as shown by *BRACHYURY* expression), trophoectoderm (as shown by *CDX2* expression), primitive endoderm (as shown by *GATA6* expression) and primitive ectoderm (as shown by *PAX6* expression; see Supplementary Figure 1). Both hESC and hiPSC clone 4 also show gradual upregulation of primitive ectoderm marker, *FGF5*, while very little expression of this marker is seen during differentiation of hiPSC clone 1 suggesting impaired differentiation towards this extra-embryonic lineage (Supplementary Figure 1).

Datos de la determinación de pluripotencialidad *in vivo* o formación de teratomas
Data of the pluripotentiality determination in vivo or teratoma formation

To prove the pluripotency of hiPSC clones, we carried out teratoma formation assays for both hiPSC clones 1 and 4 as well as control hESC. Human ESC (H9) grafted into SCID mice developed into teratomas that were restricted to the site of transplantation. Histological examination of teratomas revealed advanced differentiation of structures representative of all three embryonic germ layers (Figure 4A). Similarly, hiPSC clone 4 produced teratomas containing a diverse range of differentiated structures and examples of tissues from each germ layer, including neuroepithelium, kidney, intestine and cartilage (Figure 4B). In contrast, only one of the transplants for hiPSC clone 1 produced a small tissue growth that did not possess the characteristic heterogeneous structure of a fully differentiated teratoma (Figure 4C). The success rate of tumour formation was 75% for the human ESC, 33% for hiPSC clone 4 and 16% for hiPSC clone 1 (similar numbers of human ESC and hiPSC clones were transplanted as detailed in materials and methods).

Datos de la tipificación HLA
HLA typification data: not carried out

Consistencia celular tras 6 pases de congelación y descongelación. Resultados.
Cell consistency alter 6 passages of freezing and thawing. Results. normal

Pase en el momento del registro
Passage at the time of the recording: 60

¿Ha sido la línea modificada genéticamente? *Has the line been genetically modified?* **Sí** *Yes* ☐ **No** *No* ☒ **Comentarios/** *Comments***:**	**¿Se llevó a cabo un análisis clonal?** *Has a clonal analysis been carried out?* **Sí/** *Yes* ☐ **No** ☒ **Resultado /** *Result*

Otras observaciones o información relevantes (a juicio del Investigador Principal):
Other observations or relevant information (to the discretion of the Principal Investigator):

Three new markers, *ABCG2*, *REX1* and *DNMT3B* have been suggested as potential tools for distinguishing between fully and partially reprogrammed hiPSC colonies (14). In view of this we carried out quantitative RT-PCR analysis for expression of a range of pluripotency markers including the above three mentioned genes. In contrast to what has been described recently by Chan et al. (14) we found that *ABCG2* and *REX1* were expressed at similar levels in hiPSC clone 1 and hiPSC clone 4 when compared to human ESC (Figure 4D), hence this clone cannot be classified as partially reprogrammed. Other pluripotency markers such as *DPPA3, DPPA2, DPPA5, LEFTY2, GDF3* and *DNMT3B* were expressed at lower levels in both hiPSC clones when compared to hESC, whilst *SOX2* showed the reverse pattern. Notwithstanding this, both hESC and hiPSC clone 4 were able to downregulate the expression of all the markers investigated here during the four week time course of differentiation, whilst hiPSC clone 1 was unable to fully downregulate the expression of *ABCG2* and *REX1* and to a smaller extent the expression of *TERT, LEFTY2* and *DPPA4* (Figure 4D). For therapeutic purposes the most desirable cell lines are the hiPSC clones that can differentiate *in vitro* into cell types representative of three germ layers without forming teratomas (15). For this reason it is imperative to fully study these clones along with the ones that have the ability to give rise to teratoma and hESC. In view of this, we used both hiPSC clone 1 (non teratoma forming) and hiPSC clone 4 (teratoma forming) in all subsequent experiments shown in this manuscript.

Otras observaciones o información relevantes (a rellenar por el BNLC):
Other comments or relevant information (to be completed by BNLC)

Seguimiento de la línea (a rellenar por el BNLC):

Follow up of the line (to be completed by BNLC)

SECCIÓN 4 Declaración

Confirmo que la información contenida en estos impresos es cierta y asumo total responsabilidad sobre la misma.
I confirm that the information contained in this form is true and I assume total responsibility for it.

Firma en Representación del Centro / Signature in Representation of the Centre *(Representante legal del Departamento/Centro)* *(Legal Reprentative of the Department/Centre)* Fecha/ Date:	**Firma del Investigador Principal** *Signature of the Principal Investigator* Fecha /Date
Nombre y Cargo de la Persona Representante del Centro: *Name and Position of the Person Representing the Centre:*	
Dirección Postal: *Postal Address:*	**Teléfono /***Telephone***:** **Fax:** **E-mail:**