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 (Anexo I).
Others (specify)

SECCIÓN 1 — Información General
Section 1 — General Information

Nombre de la línea:
Name of the line:

MSUH-001

Investigador principal:
Principal Investigator:

Dr. Pablo Menéndez (BACM)

Prof. Jose Cibelli, Dr. Steve Suhr, Dr. EunAh Chang, (Michigan State University)

Origen de la línea celular:
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

The IMR90 fibroblast line is derived from a 16 week-gestation age female. Both the input IMR90 line and the resulting IPSC line MSUH-001 have a normal (and identical) karyotype as determined by pre- and post-conversion analysis.

SECCIÓN 2
Section 2

Datos del Depositante
Applicant Details

Investigador Principal: *Principal Investigator:* Pablo Menéndez Jose Cibelli, Steve Suhr, EunAh Chang,	**Dirección Postal:** *Postal address:* Cellular Reprogramming Laboratory, Michigan State University, B270 Anthony Hall, East Lansing, MI 48824 USA Banco Andaluz de Células Madre. Avda del Conocimiento s/n. PTS. 18100. Armilla. Granada. Spain
Centro de Trabajo: *Institution:* Michigan State University (with the collaboration of BACM)	**Teléfono** *(phone)***:** 517-432-7065 **Fax:** 517-432-8742 **E-mail:** cibelli@anr.msu.edu

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 derived from fetal female fibroblast line IMR90

Muestra biológica
Biological sample

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

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

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)

Fetal IMR90 fibroblasts were obtained from ATCC and cultured for several passages to achieve good healthy growth. Growing IMR90s were infected with high-titer lentiviral vectors encoding the reprogramming factors Oct4, Sox2, Nanog, and Lin28. The infected IMR90 cells were then switched into human ES-cell medium supplemented with 100ng/ml zFGF2 until the formation of ES-cell like colonies at approximately day 21-post infection. The ES-like colonies were then passaged, expanded, and maintained on blocked MEFs in human ES medium until establishment of a stable and uniform IPS cell identity. At later passages, FGF2 could be reduced to levels typical of human ESC medium (4-20ng/ml).

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

N/A

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).

Input IMR90 fibroblasts were cultured in DMEM/10%FBS/pen-strep-antimycotic, and the derived IPSCs (line MSU-001) were cultured in standard hES medium (KO-DMEM supplemented with 20% KSR, 2mmol/l glutamine, 0.05% beta-mercaptoethanol, 1% NEAA. and 0.5% pen-strep with 100ng/ml zFGF2 or 5-20ng/ml hFGF2). All reagents from Gibco-Invitrogen unless otherwise noted.

Mantenimiento de la línea: *Line maintenance*
Ratio de pase: *Passage ratio*
Método de pase: *Passage method*
IPSCs are passaged mechanically by manually selecting and picking colonies with good ES-cell like morphology and passaging approx. 1:3 onto blocked MEFs.

Xenobióticos	**si**	**no**
Xenobiotics	*Yes*	*XNo*

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)

Small, tightly packed cells with a high nucleus/cytoplasm ratio and prominent nucleoli that grow in circular colonies with defined edges.

Controles microbiológicos realizados (indicar detalladamente)

Microbiological controls carried out (indicate in detail)

Mycoplasm negative as determined by PCR.
Mycology tests and bacteriology tests negative as determined by Farmacopea Standards for sterility assays.
HLAY typing and STR studies reveal that this cell line is distinct from previous iPS cell lines derived and deposited.

Marcadores:
Markers

	Método (ARN/proteínas) *Method (RNA/proteins)*	**nº pase** *Passage n.*	**resultado** *results*	**comentarios** *comments*
Oct 4	**qPCR, immunfluorescence,**	**P5**	**+**	
Nanog	**qPCR, immunfluorescence,**	**P5**	**+**	
Sox 2	**qPCR, immunfluorescence,**	**P5**	**+**	
SSEA3	**immunfluorescence,**	**P5**	**+**	
SSEA4	**immunfluorescence,**	**P5**	**+**	
TRA-1-60	**Flow Cytometry**	**P18**	**+**	
TRA-1-81	**Flow Cytometry**	**P18**	**+**	

Telomerasa/*Telomerase* ***Telomere elongation positive by TRF analysis***
Fosfatasa Alk. */Alkaline phosphatase* ***Not done***
Cariotipo / *Karyotype* *P5* *46, XX*
Otros / *Others* ***genome-wide methylation profile, X inactivation normal, microRNA and mRNA profile, consistant with IPS phenotype***

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	**TUJ1, GFAP, HB9, GAD67, GABAR,**	**P5-7**		**AFP,**	**P5-7**		**sarc Myo, myogenein**	**P5-7**	

In vitro

Blood Differentiation: MSUH 001 lines gives rises to hemangioblasts and CD45+ blood cells in differentiation specific experiments.

In vivo/ *in vivo* **from teratoma tissue.**
Método: Teratoma formation in SCID mice, culture of immunopositive CNS cells
Method:
Resultado:
Result: +

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

Teratoma formation, culture of differentiated cells from teratoma, embryoid body formation.

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

Hemotoxylin/Eosin staining of histological sections of teratoma, immunocytochemistry of cells differentiated in vitro down multiple cell lineages and from EBs

Datos de la tipificación HLA
HLA typification data
See Annex 1, page 8.

Class I: HLA-A*0201,*0201 ; B*4001,*8201 ; Cw*0401*, *0701
Class II: HLA-DR B1*0404, 0405 ; HLA-DQB1*0302, *0302

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

Pase en el momento del registro
Passage at the time of the recording
Passage 26.

¿Ha sido la línea modificada genéticamente?
Has the line been genetically modified?

Sí *Yes* ☒ **No** *No* ☐

Comentarios/ *Comments*: **Introduction of reprogramming factors by retrovirus used to make line**

¿Se llevó a cabo un análisis clonal?
Has a clonal analysis been carried out?

Sí/ *Yes* ☐ **No** ☒ **Resultado /** *Result*
It is likely that the MSU001 IPSC line is clonal.

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

Genome-wide methylation profile has been done.
X inactivation is normal.
microRNA and mRNA profile have been done and are consistant with IPS phenotype.

(Please, see publications attached).

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)

MSUH-001 iPS cell line has been successfully maintained for over 18 passages. Importantly, this cell line is growing not only in feeders but also in matrigel-based feeders-free culture system.

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 *(Legal Representative of the Department/Centre)* **D. Juan Jesús Bandera González** Fecha/ Date: **10/03/2010**	**Firma del Investigador Principal** *Signature of the Principal Investigator* **Dr. Pablo Menéndez Buján** Banco andaluz de células madre Centro de Investigación Biomédica Parque Tecnológico de Ciencias de la Salud Avda. del Conocimiento s/n 18100 I Armilla I Granada www.bancoandaluzdecelulasmadre.es Fecha /Date: **10/03/2010**
Nombre y Cargo de la Persona Representante del Centro: *Name and Position of the Person Representing the Centre:* **D. Juan Jesús Bandera González** **Director Gerente** **Fundación Progreso y Salud**	
Dirección Postal: *Postal Address:* **Avda.Américo Vespucio 5, Bloque 2, 2ª Planta** **Parque Científico y Tecnológico Cartuja 93 - 41092** **Sevilla**	**Teléfono** */Telephone*: **955040450** **Fax: 955040457** **E-mail: gestionproyectos.fps@juntadeandalucia.es**

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Firma en Representación del Centro / Signature in Representation of the Centre *(Legal Representative of the Department/Centre)* Fecha/ Date: 02-18-2010	**Firma del Investigador Principal** *Signature of the Principal Investigator* Fecha /Date 2/24/10
Nombre y Cargo de la Persona Representante del Centro: *Name and Position of the Person Representing the Centre:* Michael R. Poterala, Executive Director of MSU Technologies RBS	
Dirección Postal: *Postal Address:* Michigan State University MSU Technologies 325 E. Grand River, Suite 350 East Lansing, MI 48823 USA	**Teléfono** */Telephone*: 1-517-355-2186 **Fax:** 1-517-432-3880 **E-mail:** poterala@msu.edu

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