Stem Cell Research 49 (2020) 102082

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab resource: Stem Cell Line

Generation, establishment and characterization of a pluripotent stem cell line (CVTTHi001-A) from primary fibroblasts isolated from a patient with activated PI3 kinase delta syndrome (APDS2)

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A B S T R A C T

APDS2 is caused by mutations in *PIK3R1* gene resulting in constitutive PI3Kδ activation. PI3Kδ is predominantly expressed in leukocytes and plays critical roles in regulating immune responses. Here we first derived fibroblast primary cells from a skin biopsy of a patient carrying a heterozygous single T deletion in intron 11 of the *PIK3R1* gene. We next present the derivation of an induced pluripotent stem cell (iPS) line using a non-integrative reprogramming technology. Pluripotent-related hallmarks are further shown, including: iPSCs self-renewal and expression of pluripotent and differentiation markers after *in vitro* differentiation towards embryonic germ layers, assessed by RT-PCR and immunofluorescence.

Resource Table:

Unique stem cell line identifier	CVTTHi001-A
Alternative name(s) of stem cell line	*Not applicable*
Institution	Basque Centre for Blood Transfusion and Human Tissues (CVTTH), Osakidetza, Biocruces Bizkaia Health Research Institute
Contact information of distributor	Cristina Eguizabal Argaiz cristina. eguizabalargaiz@osakidetza.eus
Type of cell line	iPSC
Origin	Human fibroblast
Additional origin info	22-years-old caucasian male
Cell Source	Fibroblast
Clonality	Clonal
Method of reprogramming	Transgene free (Cytotune™ iPS 2.0 Sendai reprogramming kit)
Genetic Modification	YES
Type of Modification	Hereditary
Associated disease	Activated phosphoinositide 3-kinase δ syndrome 2 (APDS2)
Gene/locus	PIK3R1/chr 5q13.1
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	26/10/2020
Cell line repository/bank	

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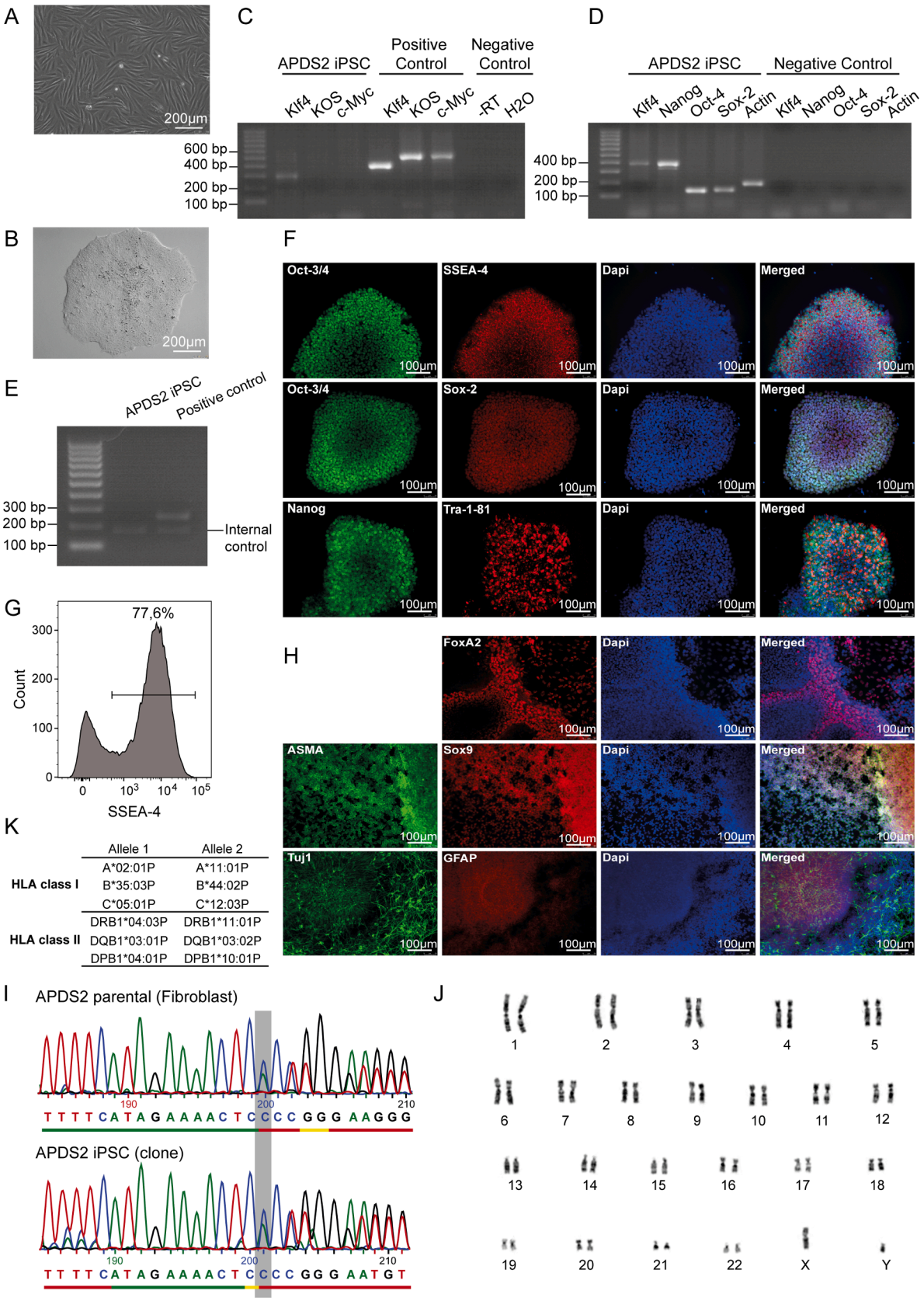
https://doi.org/10.1016/j.scr.2020.102082
Received 27 October 2020; Accepted 1 November 2020
Available online 7 November 2020



K

	Allele 1	Allele 2
HLA class I	A*02:01P	A*11:01P
	B*35:03P	B*44:02P
	C*05:01P	C*12:03P
HLA class II	DRB1*04:03P	DRB1*11:01P
	DQB1*03:01P	DQB1*03:02P
	DPB1*04:01P	DPB1*10:01P

Fig.1. Characterization of APDS2 fibroblast-derived hiPS cell line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal human iPSC colony morphology with well-defined borders	Fig. 1 panel B
Phenotype	Qualitative analysis Immunofluorescence, RT-PCR	Expression of pluripotency markers: SSEA-4, Oct-3/4, Sox-2, Tra-1-81, Nanog. Expression of endogenous pluripotency genes: Klf4, Nanog, Oct-4, Sox-2.	Fig. 1 panel F and D
	Quantitative analysis (Flow Cytometry)	Expression of SSEA-4: 77.6%	Fig. 1 panel G
Genotype	Karyotype (G-banding)	46XY without abnormalities, Resolution 550	Fig. 1 panel J
Identity	STR analysis	Performed	Supplementary file 1 ("STR analysis")
	STR analysis	16 sites tested, 16 sites matched	Submitted to the journal
Mutation analysis (IF APPLICABLE)	Sanger Sequencing	Heterozygous single nucleotide deletion (T)	Fig. 1 panel I
	Southern Blot OR WGS	*N/A*	*N/A*
Microbiology and virology	Mycoplasma	Samples tested negative by PCR	Fig. 1 panel E
Differentiation potential	*In vitro* three germ layers differentiation	Expression of differentiation markers: FoxA2 (endoderm), Sox9 and ASMA (mesoderm), Tuj1 and GFAP (ectoderm)	Fig. 1 panel H
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	*N/A*	*N/A*
	HLA tissue typing	HLA typed Class I and Class II	Fig. 1 panel K

(*continued*)

	hPSCreg repository https://hpscreg.eu/cell-line/CVTTHi001-A
Ethical approval	The study was approved by the Basque Committee of Ethics and Clinical Research (Spain). Approval number PI2014206.

1. Resource utility

The derivation and characterization of a fibroblast-derived iPS cell line from a patient with APDS2 represents an unprecedented cell culture system to model and study APDS2-related clinical pathology. Furthermore, APDS2 iPSCs may open the door for the development of new treatments, representing a unique source as drug-screening platform and a model system for genome editing technology.

2. Resource details

The APDS2 primary immunodeficiency (PID), also called "p110δ-activating mutations causing senescent T cells, lymphadenopathy and immunodeficiency (PASLI-R1)" is caused by mutations in the gene that encodes the regulatory subunit of the heterodimer PI3K, a class IA complex. Two subunits compose class IA PI3K molecules: the catalytic subunit (p110α, p110β or p110γ) and the regulatory subunit (p85α, p55α, p50α, p85β or p55γ) encoded by the *PIK3CD* and the *PIK3R1* genes, respectively. The stability, cellular localization and function of p110 are regulated by the binding of any of the regulatory subunits. The expression of catalytic subunit p110γ is restricted mainly to leukocytes and p85α is the predominant regulatory subunit in lymphocytes (Elkaim et al., 2016). Several autosomal dominant mutations in a splice donor site of *PIK3R1* gene resulting in an in-frame-skipping of exon 11 (coding exon 10) can cause APDS2 (Michalovich and Nejentsev, 2018).

In this study, a human iPSC-line was established from primary fibroblasts isolated from a skin biopsy of a 22-years-old patient carrying a heterozygous single-nucleotide T deletion at position + 2 (c.1425 + 2delT) of the *PIK3R1* gene splice donor site. This deletion results in the generation of a shorten transcript demonstrated by mRNA analysis by RT-PCR (data not shown), and consequently in a reduced regulatory subunit protein that lacks coding exon 10 (p.434-475del).

Primary fibroblast cell cultures from ADPS2 patient (Fig. 1A) were induced to pluripotency using the non-integrating CytoTune-iPS 2.0 Sendai Reprogramming kit (Invitrogene#A16517) containing Oct-4, Klf4, Sox-2 and c-Myc pluripotency transcription factors following the manufacturer's instructions. The resulting APDS2-iPSC line (registered as CVTTHi001-A at the Human Pluripotent Stem Cell Registry, http://hPSCreg.eu) grew exhibiting the typical colony shape morphology (Fig. 1B). After some culture passages using mechanical disaggregation, expression of pluripotent genes was assessed by RT-PCR. As shown in Fig. 1C, exogenous transgene expression was silenced in APDS2-iPSC line while expression of endogenous pluripotent genes was detected (Fig. 1D) collectively demonstrating that the pluripotent state was not transitory. Assessment of mycoplasma amplification was negative (MinervaBiolabs#111050) (Fig. 1E). APDS2-iPSC line was assayed for the detection of protein expression for the transcription factors Oct-3/4, Nanog and Sox-2, and the surface markers Tra-1-81, SSEA-4 by immunofluorescence and flow cytometry analysis (Fig. 1F, G). Moreover, APDS2-iPSC line was further differentiated towards the three germ layers of the embryo following the manufacturer's instructions of the STEMDiff kit (STEMCEL#05230). After differentiation induction, immunofluorescence analysis showed the detection of markers of endoderm, mesoderm and ectoderm fates as indicated in Fig. 1H, corroborating the inherent capacity of APDS2-iPSC line to differentiate *in vitro*. Sanger sequencing was used to further verify the detection of the heterozygous single-nucleotide T deletion at position +2 in c.1425 of the *PIK3R1* gene (Table1, Fig. 1I). Karyotype analysis further confirmed the absence of major chromosomal aberrations in APDS2-iPSC line (Fig. 1J). Genotype of HLA (human leukocyte antigen) genes of APDS2-iPSC line are shown in Fig. 1K. All in all our approach sufficed for the generation of ADPS2 iPSC-line representing an affordable cell source to further study APDS2 etiology and pathology. Identity of donor fibroblasts and its derived iPSC line was tested by STR analysis (Supplementary file 1).

3. Materials and methods

3.1. Reprogramming and maintenance of the ADPS2 iPSC-line

The minced patient skin biopsy was treated overnight at 37 °C with 7500U of collagenase IV (LifeTechnologies#17104019) in 5 mL of DMEM (ThermoFisher#11965-092) supplemented with 10% FBS (ThermoFisher#10270-106), 1 mM Glutamax (LifeTechnologies#35050-038) and 50 U/mL of penicillin/streptomycin (LifeTechnologies#15140-122) [DMEM-FBS]. Following overnight collagenase treatment, the suspension was filtered through a 40 μm cell strainer and washed two times with DMEM-FBS medium. Fibroblast were left to grow into 10 cm culture dishes until 80% confluence and further expanded in DMEM-FBS medium. Reprogramming was performed following the manufacturer's instructions of the CytoTune-iPS

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency	Rabbit anti-Oct-3/4	1:25	Santa Cruz Biotechnology Cat# sc 9081, RRID:AB_2167703
Pluripotency	Mouse anti-Oct-3/4	1:25	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
Pluripotency	Mouse anti-SSEA4	1:03	Hybridoma Bank Cat# MC-813-70, RRID:AB_528477
Pluripotency	Goat anti-Sox-2	1:25	Santa Cruz Biotechnology Cat# sc-17320, RRID:AB_2286684
Pluripotency	Goat anti-Nanog	1:25	R&D Systems Cat# AF1997, RRID:AB_355097
Pluripotency	Mouse anti -Tra-1-81	1: 200	Millipore Cat# MAB4381, RRID:AB_177638
Differentiation (Endoderm)	Goat anti-FoxA2	1:25	R&D Systems Cat# AF2400, RRID:AB_2294104
Differentiation (Ectoderm)	Mouse anti-Tuj1	1:500	Covance Cat# MMS-435, RRID:AB_2313773
Differentiation (Ectoderm)	Rabbit anti-GFAP	1:1000	Abcam. Cat# ab7260, RRID:AB_305808
Differentiation (Mesoderm)	Mouse anti-ASMA	1:400	Sigma-Aldrich Cat# A5228, RRID:AB_262054
Differentiation (Mesoderm)	Rabbit anti-Sox9	1:300	Sigma-Aldrich Cat# AB5535, RRID:AB_223976
Pluripotency	Alexa Fluor 488 donkey anti-goat IgG	1:200	ThermoFisher Scientific Cat# A11055, RRID:AB_2534102
Pluripotency	Alexa Fluor 546 donkey anti-mouse IgG	1:200	ThermoFisher Scientific Cat# A10036, RRID:AB_2534012
Pluripotency/Differentiation	Alexa Fluor 488 donkey anti-rabbit IgG	1:200	ThermoFisher Scientific Cat# A21206, RRID:AB_2535792
Pluripotency/Differentiation	Alexa Fluor 488 donkey anti-mouse IgG	1:200	ThermoFisher Scientific Cat# R37114, RRID:AB_2556542
Pluripotency/Differentiation	Alexa Fluor 546 donkey anti-goat IgG	1:200	ThermoFisher Scientific Cat# A11056, RRID:AB_142628
Pluripotency	Cy™3 AffiniPure donkey anti-mouse IgM	1:200	Jackson ImmunoResearch Cat# 715-165-140, RRID:AB_2340812
Differentiation	Alexa Fluor 546 donkey anti-rabbit IgG	1:200	ThermoFisher Scientific Cat# A10040, RRID:AB_2534016
Pluripotency (flow cytometry)	PE Mouse anit-SSEA-4	20 µl	BD Biosciences Cat# 560128, RRID: AB_1645533
Primers			
	Target	**Forward/Reverse primer (5′-3′)**	
Virus detection marker RT-PCR	SeV (181 bp)	GGATCACTAGGTGATATCGAGC / ACCAGACAAGAGTTTAAGAGATATGTATC	
Virus detection marker RT-PCR	KOS (528 bp)	ATGCACCGCTACGACGTGAGCGC / ACCTTGACAATCCTGATGTGG	
Virus detection marker RT-PCR	Klf4 (410 bp)	TTCCTGCATGCCAGAGGAGCCC / AATGTATCGAAGGTGCTCAA	
Virus detection marker RT-PCR	c-Myc (532 bp)	TAACTGACTAGCAGGCTTGTCG / TCCACATACAGTCCTGGATGATGATG	
Pluripotency marker RT-PCR	Oct-4 (144 bp)	GACAGGGGGAGGGGAGGAGCTAGG / CTTCCCTCCAACCAGTTGCCCCAAAC	
Pluripotency marker RT-PCR	Sox-2 (151 bp)	GGGAAATGGGAGGGGTGCAAAAGAGG / TTGCGTGAGTGTGGATGGGATTGGTG	
Pluripotency marker RT-PCR	Klf4 (397 bp)	ACGATCGTGGCCCCGGAAAAGGACC / TGATTGTAGTGCTTTCTGGCTGGGCTCC	
Pluripotency marker RT-PCR	Nanog (343 bp)	CAGCCCCGATTCTTCCACCAGTCCC / CGGAAGATTCCCAGTCGGGTTCACC	
Housekeeping gene RT-PCR	β-Actin (194 bp)	TCCCTGGAGAAGAGCTACGA / AGCACTGTGTTGGCGTACAG	
Deletion detection Sequencing	PIK3R1 (469 bp)	**CTAG**GATGTTTCCATGTCAGC / GAAACTCAGTGACTGCTTCAG	

2.0 Sendai Reprogramming kit (Invitrogene#A16517). Cells were transduced at 30–60% confluence with viral particles with MOIs (multiplicity of infection) of KOS (Klf4, Oct-4 and Sox-2), hc-Myc and hKlf4 of 5, 5 and 3 respectively. At day 7, cells were seeded in vitronectin-(Gibco#A31804) coated 6-well-plates at different dilutions (25.000 to 300.000 cells) and cultured with Essential 8 Medium (Gibco#A1517001). Undifferentiated ADPS2-derived colonies were transferred onto vitronectin-coated culture dishes at day 18 of the reprogramming process. Five ADPS2-derived iPSC lines were passaged and amplified by mechanical dissection of undifferentiated fragments and transferred to freshly vitronectin-coated dishes for the different assays detailed hereafter.

3.2. RT-PCR

Total RNA was extracted using RNAqueous Micro Kit (Ambion#AM1931). Following DNase treatment, RNA was retrotranscribed to cDNA using Super Script VILO kit (Invitrogen#11754050). RT-PCR was performed using primers for amplification of pluripotent and reprogramming genes as indicated in Table 2. Primer sequences for pluripotency markers and β-Actin were obtained from Cabrera et al. (2015) and Yang et al. (2015) respectively.

3.3. Immunofluorescence

ADPS2 colonies grown at 80% of confluence were fixed in 4% paraformaldehyde for 30 min at room temperature (RT) and washed 3 times with 1X Tris-Buffered Saline (TBS) (50 mM Tris-Cl, pH 7.6; 150 mM NaCl) for 5 min. Permeabilization and blocking was performed with TBS-T [TBS 1X + 0.5% Triton X-100 (Sidgma-Aldrich#9002-93-1)] with 6% of donkey serum (Millipore#S30-100ML) for 30 min at RT. Primary antibodies were incubated overnight (Oct-3/4, Sox-2, SSEA-4, Nanog, Tra-1-81) or during 48 h (AFP, FoxA2, GFAP, Tuj1, ASMA, Sox9) at 4 °C. After three washes with 1X TBS, corresponding secondary antibodies were added for 2 h at 37 °C. Nuclei were counterstained with 0.1 µg/mL DAPI (LifeTechnologies#D21490). Images were taken with 20X objective of the Leica Microsystems DMi8 inverted microscope.

3.4. Sequencing

Primers were designed 200–250 nucleotides away from the deletion. Due to a high concentration of adenosines and thymidines in the sequence of interest, the forward primer was modified in its 5′ end by the addition of a 4-nucleotide adapter (shown in bold in Table 2). Following DNA isolation, PCR-amplified DNA fragments were sequenced by Eurofins Genomics Company.

3.5. Karyotype

Karyotype analysis was performed on iPSC cells in passage 24 by G-banded metaphase spreads that were prepared according to standard protocol of the Genetic Unit of Hospital de Basurto (Spain). Twenty metaphases images were taken and processed with GSL 120 Leica Microsystems.

3.6. Mycoplasma detection

Mycoplasma detection was performed with over-weekend cell culture medium following the protocol of the mycoplasma detection kit (MinervaBiolabs#11-1050).

3.7. STR analysis

Genetic identity of CVTTH001-A was determined by profiling of STR loci using the AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit (AppliedBiosystems#4427368) by the Genomic Platform of CIC bioGUNE (Spain).

3.8. Flow cytometry

Cells were detached using 0.5 mM EDTA and assessed for viability (Invitrogen#L34975) and SSEA-4 expression.

3.9. HLA characterization

HLA determination was performed in CVTTH (Galdakao, Spain) using next generation sequencing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by Health Department of Basque Government (Grant 2016111140) and Project "PI18/01299", funded by Instituto de Salud Carlos III and co-funded by European Union (ERDF) "A way to make Europe". M.I-F was supported by Inocente Inocente Foundation (FII18-003-CPS). M.M-I was supported by Jesus Gangoiti Barrera Foundation and by Health Department of Basque Government (Grant 2020333032). L.H was supported by Jesus Gangoiti Barrera Foundation, Asociación Española contra el Cáncer (AECC) and Fundación mutua Madrileña. E.G. was supported by the EFSD/Boehringer Ingelheim European Research Programme in Microvascular Complications of Diabetes and by the EIT Health under grant ID 20366 (R2U-Tox-Assay).

References

Cabrera, Sonia, Ji, Ae-Ri, Frejo, Lidia, Ramos-Mejia, Veronica, Romero, Tamara, Real, Pedro, Lopez-Escamez, Jose A., et al., 2015. Generation of human iPSC line GRX-MCiPS4F-A2 from adult peripheral blood mononuclear cells (PBMCs) with Spanish genetic background. Stem Cell Res. https://doi.org/10.1016/j.scr.2015.07.002.

Elkaim, E., Neven, B., Bruneau, J., Mitsui-Sekinaka, K., Stanislas, A., Heurtier, L., Lucas, C.L., Matthews, H., Deau, M.-C., Sharapova, S., Curtis, J., Reichenbach, J., Glastre, C., Parry, D.A., Arumugakani, G., McDermott, E., Kilic, S.S., Yamashita, M., Moshous, D., Lamrini, H., Otremba, B., Gennery, A., Coulter, T., Quinti, I., Stephan, J.-L., Lougaris, V., Brodszki, N., Barlogis, V., Asano, T., Galicier, L., Boutboul, D., Nonoyama, S., Cant, A., Imai, K., Picard, C., Nejentsev, S., Molina, T.J., Lenardo, M., Savic, S., Cavazzana, M., Fischer, A., Durandy, A., Kracker, S., 2016. Clinical and immunologic phenotype associated with activated phosphoinositide 3-kinase δ syndrome 2: a cohort study. J. Allergy Clin. Immunol. 138 (1), 210–218.e9. https://doi.org/10.1016/j.jaci.2016.03.022.

Michalovich, D., Nejentsev, S., 2018. Activated PI3 kinase delta syndrome: from genetics to therapy. Front. Immunol. 27 (9), 369. https://doi.org/10.3389/fimmu.2018.00369. PMID: 29535736; PMCID: PMC5835040.

Yang, Qingqing, Zhou, Xiaoying, Zhou, Hao, Li, Long, Du, Juan, Lu, Guangxiu, Lin, Ge, Ouyang, Qi, 2015. Human embryonic stem cells derived from abnormal blastocyst donated by Marfan syndrome patient. Stem Cell Research. https://doi.org/10.1016/j.scr.2015.10.012.