



**Generation of three Duchenne Muscular Dystrophy  
patient-specific induced pluripotent 4 stem cell lines  
DMD\_YoTaz\_PhyMedEXp, DMD\_RaPer\_PhyMedEXp, 5  
DMD\_OuMen\_PhyMedEXp (INSRMi008-A, INSRMi009-A and  
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# Generation of three Duchenne Muscular Dystrophy patient-specific induced pluripotent stem cell lines DMD\_YoTaz\_PhyMedEXp, DMD\_RaPer\_PhyMedEXp, DMD\_OuMen\_PhyMedEXp (INSRMi008-A, INSRMi009-A and INSRMi010-A)

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## A B S T R A C T

Duchenne Muscular Dystrophy (DMD) is a X-linked degenerative pathology with a prevalence of 1/3600–6000 boys due to the absence of functional dystrophin in muscles. This muscular disease leads to skeletal muscle damages, respiratory failure and in the later stages dilated cardiomyopathy (DCM) leading to heart failure. We generated iPSC lines from three different DMD patients carrying respectively deletions of exons 1, 52 and 55 in the dystrophin gene. The reprogrammed iPSC lines showed expression of pluripotent markers, capacity to differentiate in trilineage embryonic layers and a normal karyotype.

### 1. Resource table

Unique stem cell lines identifier	1. INSRMi008-A; 2. INSRMi009-A; 3. INSRMi010-A
Alternative names of stem cell lines	1. DMD_YoTaz_PhyMedExp 2. DMD_RaPer_PhyMedExp 3. DMD_OuMen_PhyMedExp
Institution	SAFE-iPSC Facility INGESTEM, CHU of Montpellier
Contact information of distributor	Albano C. Meli (albano.meli@inserm.fr)
Type of cell lines	iPSC
Origin	human
Cell Source	Blood
Clonality	mixed
Method of reprogramming	Sendai virus kit 2.0 expressing Klf4–Oct3/4–Sox2 (KOS), c-Myc.
Multiline rationale	Mutation in DMD gene with deletion of exons 1, 52 and 55
Gene modification	YES
Type of modification	Hereditary
Associated disease	Duchenne Muscular Dystrophy

(continued on next column)

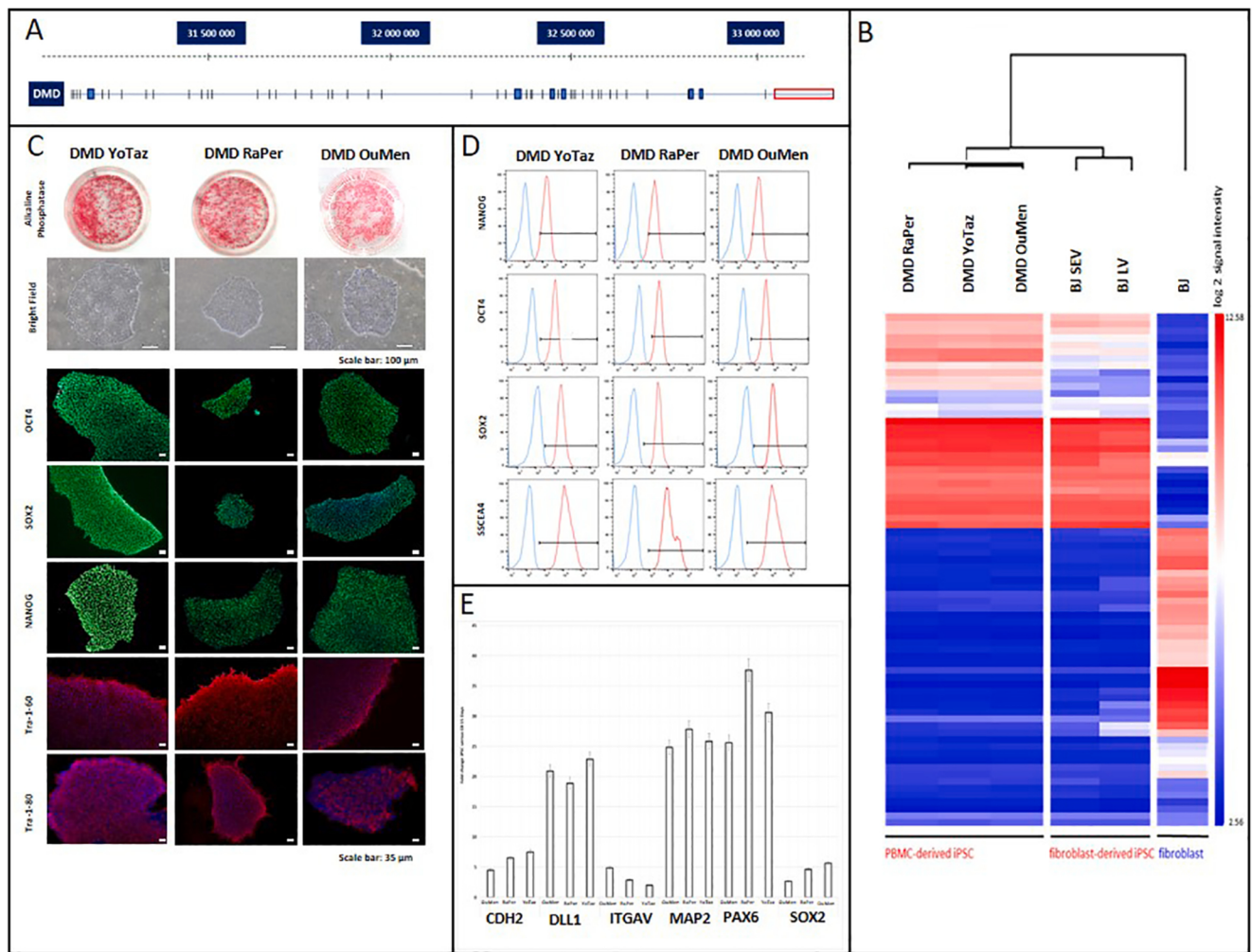
(continued)

Gene/locus	Xp21
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	N/A
Cell line repository/bank	SAFE-iPSC facility IRMB
Ethical approval	CPP Ouest 6-CPP 1003-HPS2 CHU MONTPELLIER

### 2. Resource utility

DMD leads to DCM and heart failure which cause premature death. We generated iPSC lines from 3 DMD patients to obtain iPSC derived cardiomyocytes (iPSC-CMs) allowing us to decipher the molecular and pathophysiological mechanisms causing aberrant cellular changes in DMD iPSC-CMs and to test drugs to treat DMD-associated cardiac disease.

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**Fig. 1. DMD induced pluripotent stem cell lines generated by reprogramming of PBMC from patients with the following mutations DMD RaPer, DMD YoTAZ, DMD OuMen** A – **DMD gene** spans a genomic range of greater than 2 Mb on chromosome X (chrX:3119222-32155469). The deletion shown by the red box is causing the Duchenne muscular dystrophy (DMD). B - **Heatmap of DMD iPSCs lines transcriptome analyzed by supervised clustering on genes involved in pluripotency**: List of genes involved in pluripotency and transcriptomic analysis performed on iPS derived with the sendai virus from DMD RaPer, DMD YoTAZ, DMD OuMen patients and iPS derived from BJ fibroblast (BJ SEV), and with a lentivirus (BJ LV), and the BJ fibroblast obtained from the Coriell Institute. C - **Characterization of DMD iPSC colonies** by alkaline phosphatase staining and morphology (bright field picture), and by Immunofluorescence staining after 10 passages analyzed by microscopy using specific pluripotency markers from top to bottom OCT4, SOX2, NANOG, TRA-1-60 and TRA1-80. D – **Analysis of the expression of pluripotency markers by flow cytometry for the DMD iPSCs lines** for NANOG, OCT4 and TRA-1-60 and TRA1-80. A negative control is shown with blue line and DMD iPSC cell line staining are shown on the same graphic by the red line. E – **Gene expression** increase between iPSC cell line (DMD RaPer, DMD YoTAZ, DMD OuMen) and Embryoid Bodies for genes known to be expressed during differentiation.

### 3. Resource details

DMD is a X linked disease caused by the absence of functional dystrophin due to nonsense mutations. DMD in the most common and severe form of muscular dystrophy and occurs in 1/3600–6000 male births (Birnkrant et al., 2018; Koenig et al., 1989). This pathology is associated with severe skeletal muscle wasting, respiratory failure and often with DCM leading to premature death (Finsterer and Stollberger, 2007; Phillips et al., 2001). The conventional markers of DMD are insufficient to perform an early diagnosis of this cardiac disorder. Finding new and early markers of DMD-associated DCM is of great clinical interest.

Moreover, it is very difficult to obtain cardiac tissue from the patient for ethical reasons. While the animal models are not always pertinent to decipher the molecular mechanisms of human cardiac disorders, the iPSC-CMs emerge as an exciting and complementary tool to characterize the phenotype and evaluate the degree of severity of the disease in a patient-specific context in a dish. To investigate the DMD-associated DCM, we generated patient-specific iPSC lines that were subsequently differentiated into cardiomyocytes. Following institutional ethical committee approval and patient informed consent, peripheral blood mononuclear cells (PBMC) were isolated by blood samples obtained from three independent DMD boys (9, 12 and 14 years-old) harboring

**Table 1**  
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
INSRMI008-A	DMD YoTAZ	Male	9	N/A	DMD	DMD
INSRMI009-A	DMD RaPer	Male	12	N/A	DMD	DMD
INSRMI010-A	DMD OuMen	Male	14	N/A	DMD	DMD

**Table 2**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	<a href="#">Fig. 1 Panel C</a>
Phenotype	Qualitative analysis (i.e. Immunocytochemistry)	Assess staining/expression of pluripotency markers: Oct4, Sox2, NANOG, Tra-1–60, Tra-1–80	<a href="#">Fig. 1 Panel C</a>
	Quantitative analysis (i.e. Flow cytometry)	Assess % of positive cells or transcripts for antigen & cell surface markers DMD YoTaz: NANOG: 100% OCT4: 100% SOX 2: 100% SSCEA4: 100% DMD RaPer: NANOG: 100% OCT4: 100% SOX 2: 100% SSCEA4: 100% DMD OuMen: NANOG: 100% OCT4: 100% SOX 2: 100% SSCEA4: 100%	<a href="#">Fig. 1 panel D</a>
Genotype	Karyotype (G-banding) and resolution (CGH array)	46XX, Resolution 450–500	Supplementary <a href="#">Fig. 1</a>
Identity	STR analysis	DNA Profiling performed with 17 sites, matched	Supplementary file 1 submitted in archive with journal
Mutation analysis	MLPA	X-linked mutation on exons: 1 (DMD_YoTaz_PhyMedExp);	Supplementary file 2
Microbiology and virology	Confirmation technique: Multiplex PCR	55 (DMD_RaPer_PhyMedExp)	
	Mycoplasma	52 (DMD_OuMen_PhyMedExp)	
Differentiation potential	Embryoid body	MycoAlert® Detection Kit Negative	Not shown but available
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Ectodermal markers (CDH2, MAP2, PAX6, SOX2), endodermal markers (CDH2, PAX6) and mesodermal markers (CDH2, DLL1, ITGAV) expression showed by RT-qPCR	<a href="#">Fig. 1 Panel E</a>
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	HLA typed Class I and Class II	N/A

**Table 3**  
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry		Dilution	Company Cat # and RRID
Antibody			
Pluripotency Markers			
Flow cytometry	Oct-4A Rabbit mAb (Clone C30A3) IgG	1:200	Cell Signaling Technology Cat# 2840, RRID:AB_2167691
Pluripotency Markers			
Flow cytometry	Sox2 XP® Rabbit mAb (Clone D6D9) IgG	1:200	Cell Signaling Technology Cat# 3579, RRID:AB_2195767
Pluripotency Markers			
Flow cytometry	Nanog XP® Rabbit mAb (Clone D73G4) IgG	1:200	Cell Signaling Technology Cat# 4903, RRID:AB_10559205
Pluripotency Markers			
Flow cytometry	SSEA4 Mouse mAb (Clone MC813) IgG3	1:200	Cell Signaling Technology Cat# 4755, RRID:AB_1264259
Pluripotency Markers			
Flow cytometry	TRA-1–60(S) Mouse mAb (Clone TRA-1–60(S)) IgM	1:200	Cell Signaling Technology Cat# 4746, RRID:AB_2119059
Pluripotency Markers			
Flow cytometry	TRA-1–81 Mouse mAb (Clone TRA-1–81) IgM	1:200	Cell Signaling Technology Cat# 4745, RRID:AB_2119060
Pluripotency Markers			
Immunostaining	PE Mouse anti-human Nanog (Clone: N31-355)	1:5	BD Biosciences Cat# 560791, RRID:AB_1937305
Pluripotency Markers			
Immunostaining	PerCP-CyTM 5.5 Mouse anti-Oct3/4 (Clone: 40/Oct-3)	1:5	BD Biosciences Cat# 560794, RRID:AB_1937313
Pluripotency Markers			
Immunostaining	Alexa FluorR 647 Mouse anti-Sox2 (Clone: 245610)	1:5	BD Biosciences Cat# 560301, RRID:AB_1645308
Pluripotency Markers			
Immunostaining	Alexa FluorR 647 Mouse anti-SSEA-4 (Clone: MC813-70)	1:5	BD Biosciences Cat# 560796, RRID:AB_2033991
Pluripotency Markers			
Immunostaining	PE Mouse IgG1, κ Isotype Control (Clone MOPC-21)	1:5	BD Biosciences Cat# 554121, RRID:AB_395252
Pluripotency Markers			
Immunostaining	PerCP-Cy5.5 Mouse IgG1, κ Isotype Control (Clone: X40)	1:5	BD Biosciences Cat# 347202, RRID:AB_400265
Pluripotency Markers			
Immunostaining	Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (Clone: MOPC-173)	1:5	BD Biosciences Cat# 558020, RRID:AB_396989
Pluripotency Markers			
Immunostaining	Secondary Antibody Alexa Fluor® 488 conjugate Goat anti-Rabbit IgG	1:400	Invitrogen-Thermo Fisher Scientific Cat# A-11034, RRID:AB_2576217
Pluripotency Markers			
Immunostaining	Secondary Antibody Alexa Fluor® 555 conjugate Goat anti-Rabbit IgG	1:400	Invitrogen-Thermo Fisher Scientific Cat# A-21424, RRID:AB_141780
Primers			
Target		Forward/Reverse primer (5'–3')	
Homo sapiens cadherin 2 (CDH2)		AGCCAACTTAACTGAGGAGT/ GGCAAGTTGATTGGAGGGATG	
Homo sapiens delta-like 1 (DLL1)		GATTCTCCTGATGACCTCGCA/ TCCGTAGTAGTGTCGTGACA	
Homo sapiens integrin, alpha V (ITGAV)		GGCTGCATATTCGGATTTCGTG/ CCATTACAGCTTTGTCTCTGG	
Homo sapiens microtubule-associated protein 2 (MAP2)		TGGTGCCGAGTGAGAAGAAG/ AGTGGTTGGTTAATAAGCCGAAG	
Homo sapiens paired box 6 (PAX6)		AACGATAACATACCAAGCGTGT/ GGTCGCCCGTTCAACATC	
Homo sapiens SRY (sex determining region Y)-box 2 (SOX2)		TGGACAGTTACGCGCATAT/ CGAGTAGGACATGCTGTAGGT	



deletions in DMD gene respectively in 1, 52 and 55 exons as represented in Fig. 1A. PBMC were submitted to a transient expression of the four reprogramming factors OCT3/4, SOX2, KLF4, and C-MYC using the integration-free Sendai virus gene-delivery method. The resulting DMD iPSC were alkaline phosphatase positive and presented a normal morphology by bright field microscopy (Fig. 1C). The 3 iPSC lines are summarized in Table 1. DMD iPSC expressed pluripotency markers revealed by immunocytochemistry staining (Fig. 1C) and FACS experiments (Fig. 1D). The expression of pluripotency markers was also verified by transcriptomic analysis represented as a heatmap. The gene expression profiles were performed on DMD\_YoTaz\_PhyMedExp, DMD\_RaPer\_PhyMedExp, DMD\_OuMen\_PhyMedExp, and fibroblast-derived iPSC (Fig. 1B). The pluripotency was assessed by the ability of DMD iPSC to differentiate into the three germ layers by the formation of embryoid bodies (EBs). The resulting DMD EBs expressed specific ectoderm, endoderm and mesoderm markers revealed by RT-qPCR (Fig. 1E). The characterization of the iPSC lines is summarized in Table 2. The DMD iPSC presented no major genomic aberrations after analysis through CGH array.

## 4. Materials and methods

### 4.1. Reprogramming PBMC into iPSCs

PBMC were isolated from blood and were cultured in SFEM II (StemSpan™ SFEM II Stemcell, cat# 09605) medium with cytokines (StemSpan™ Erythroid Expansion Supplement (100X) Stemcell, Catalog cat#02692) as previously described (Gatinois et al., 2019) (Fig. 1C). The PBMC were reprogrammed using the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, cat#A34546) containing the Klf4, Oct3/4, Sox2 and c-Myc vectors, similarly to previously described (Gatinois et al., 2019).

### 4.2. iPSC culture and genomic DNA extraction

iPSC cells were plated on extracellular matrix Matrigel (Fisher Scientific, no. 354277) and cultured in Essential 8™ culture media (Thermo Fisher Scientific, no. A15169-01) as previously described (Gatinois et al., 2019). DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen) as previously described (Gatinois et al., 2019).

### 4.3. Flow cytometry analysis

BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis. Results are presented in Fig. 1D and antibodies used are in Table 3.

### 4.4. Immunofluorescence

StemLight™ Pluripotency Antibody Kit (Cell Signaling, no. 9656). Antibodies panel, listed in Table 3, included: Oct-4A, Sox2, Nanog, SSEA4, TRA-1-60, TRA-1-81.

### 4.5. CGH array

Genomic DNA extracted from the iPSC line at passage 10 and was analyzed by ChromoStem facility of Montpellier (website: <https://www.chu-montpellier.fr/fr/plateformes-recherche/chromostem>) using the chromosomal microarray was performed using SurePrint G3 Human CGH Microarray Kit, 8 × 60 K with overall median probe 41 Kb genome-wide median probe spacing. Experiments were conducted according manufacturer's protocol. Commercial reference DNA (male and female) provided by Agilent Technologies were used for the analysis. Known genomic alterations were detected. Of note, CGH does not detect translocations or inversions, alterations in chromosome structure, mosaicism or polyploidy.

### 4.6. Embryoid body formation

The iPSC were differentiated via EB formation using AggreWell™ EB Formation Medium (Stem Cell, no. 05893) and AggreWell 800 plates (Stem cell, no. 34811). Once formed, they were transferred in non-adherent 6 well plates Corning (Stem Cell, no. 3471). EB were kept for 15 days in culture.

### 4.7. RT-PCR

RNA was extracted with the Qiagen RNA easy kit following kit instruction (no. 74104). Fold increase was calculated using the algorithm, also known as the delta-delta-Ct. The level of expression of genes in EB after 15 days of differentiation was compared to their level of expression in the original iPSC as a referent sample. Sequence of these primers are in Table 3.

### 4.8. Transcriptomic analysis

Transcriptomic analysis was performed following the manufacturer's instructions as previously detailed (Gatinois et al., 2019) and shown in Fig. 1B.

### 4.9. Short tandem repeat analysis (STR)

STR analysis was performed with GeneMarker V2.6.7 (SoftGenetics) on 17 STR as previously described (Gatinois et al., 2019).

### 4.10. Mycoplasma detection

Mycoplasma is detected with MycoAlert® Detection Kit (Lonza) according to manufacturer's instructions.

## 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.

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