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Lab Resource: Multiple Cell Lines

## Generation of two isogenic induced pluripotent stem cell lines from a 4-month-old severe nemaline myopathy patient with a heterozygous dominant c.553C > A (p.Arg183Ser) variant in the ACTA1 gene

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### ABSTRACT

Nemaline myopathy (NM) is a congenital myopathy typically characterized by skeletal muscle weakness and the presence of abnormal thread- or rod-like structures (nemaline bodies) in myofibres. Pathogenic variants in the skeletal muscle alpha actin gene, *ACTA1*, cause approximately 25% of all NM cases. We generated two induced pluripotent stem cell lines from lymphoblastoid cells of a 4-month-old female with severe NM harbouring a dominant variant in *ACTA1* (c.553C > A). The isogenic lines displayed characteristic iPSC morphology, expressed pluripotency markers, differentiated into cells of all three germ layers, and possessed normal karyotypes. These lines could be useful models of human *ACTA1* disease.

### 1. Resource Table:

Unique stem cell lines identifier	1. HPIi001-A 2. HPIi001-B
Alternative names of stem cell lines	1. iPS-6303-C6B3 2. iPS-6303-R12
Institution	Harry Perkins Institute of Medical Research
Contact information of distributor	Dr. Joshua Clayton joshua.clayton@perkins.org.au
Type of cell lines	iPSC
Origin	Human
Cell Source	EBV-immortalized lymphoblastoid cell line (LCL)
Clonality	Clonal
Method of reprogramming	Sendai virus
Multiline rationale	Isogenic clones
Gene modification	Yes
Type of modification	Spontaneous variant
Associated disease	

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Unique stem cell lines identifier	1. HPIi001-A 2. HPIi001-B
Gene/locus	Nemaline myopathy 3; NEM3 (OMIM#161800), severe form Actin Alpha 1, Skeletal Muscle ( <i>ACTA1</i> ), NM_001100: c.553C > A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	September 2020
Cell line repository/bank	1. <a href="https://hpscereg.eu/cell-line/HPIi001-A">https://hpscereg.eu/cell-line/HPIi001-A</a> 2. <a href="https://hpscereg.eu/cell-line/HPIi001-B">https://hpscereg.eu/cell-line/HPIi001-B</a>
Ethical approval	Ethics approval was obtained from the Comité de Protection des Personnes (Est IV DC-2012-1693), and national consent forms for genetic testing, banking and research were signed by the patients or their legal

(continued on next page)

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E-mail address: [joshua.clayton@perkins.org.au](mailto:joshua.clayton@perkins.org.au) (J.S. Clayton).

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(continued)

Unique stem cell lines identifier	1. HPIi001-A 2. HPIi001-B
	guardian. The patient's LCLs were banked by Genethon; activity authorization No. AC-2018-3156, import/export authorization No. IE-2018-994. The study was approved by the University of Western Australia's Human Research Ethics Committee (approval number: RA/4/20/1008).

## 2. Resource utility

There are currently few tractable cell or animal models that can be used to model disease pathobiology and/or test treatments for *ACTA1* congenital myopathies. The patient-derived iPSC lines described here will complement existing *Acta1* mouse and zebrafish models and enable evaluation of genomic therapies for nemaline myopathy.

## 3. Resource details

Nemaline myopathy (NM) is a form of congenital myopathy typically characterized by muscle weakness and the presence of abnormal thread- or rod-like structures in myofibres on histologic examination (Sewry et al., 2019). Dominant variants in the skeletal muscle alpha-actin gene (*ACTA1*) are one of the most common causes of NM (Nowak et al., 1999; Sewry et al., 2019). Several recessive *ACTA1* variants are also known to cause NM (Sewry et al., 2019). Variants in *ACTA1* typically cause severe NM, which presents at birth with hypotonia and profound muscle weakness. Patients have few spontaneous movements with difficulties swallowing and sucking (Sanoudou and Beggs, 2001). Affected infants typically die from respiratory insufficiency or pneumonia within the first months of life (Sanoudou and Beggs, 2001). As such, patient samples for cell reprogramming can be difficult to obtain. The iPSC lines presented here were generated from a lymphoblastoid cell line (LCL) from a 4-month-old female with severe NM (Table 1). The patient showed neonatal disease onset, including hypotonia and hydramnios, and required ventilatory support. Genetic testing revealed the patient was heterozygous for a pathogenic variant in *ACTA1*; c.553C > A, p.Arg183Ser (Sparrow et al., 2003).

LCLs were reprogrammed into iPSCs using the CytoTune™-iPS 2.0 Sendaviruses reprogramming system. Clones were selected and expanded in mTESR™ 1 culture medium and characterized at passage 10. Both iPSC clones (HPIi001-A, HPIi001-B) had normal morphology; colonies were tightly packed and had defined edges with little to no spontaneous differentiation (Fig. 1A). Pluripotency was confirmed by qRT-PCR (Fig. 1B) and immunocytochemistry (ICC) (Fig. 1C, D). Specifically, iPSCs were enriched for *OCT4*, *SOX2*, *NANOG* and *CRIPTO* by qPCR compared to parental LCLs (Fig. 1B), and stained positively for *OCT4*, *SOX2*, *SSEA4* and *TRA-1-60* by ICC (Fig. 1C, D). Trilineage differentiation potential was assessed by directed differentiation followed by qRT-

PCR (Fig. 1E) and ICC (Fig. S1A, B). Differentiated mesoderm cultures were enriched for *TBXT* and *BMP4* by qPCR, and Brachyury (*TBXT*) by ICC. Ectoderm cultures were enriched for *OTX2* and *PAX6* by qPCR, and *OTX2* by ICC. Endoderm cultures were enriched for *GATA4* and *SOX17* by qPCR, and *GATA4* by ICC. Parental (undifferentiated) iPSC cultures showed no or negligible expression of each germ layer marker (Fig. 1E).

Both iPSC clones were confirmed to be EBV- and SeV-free at passage 10 by PCR and RT-PCR, respectively (Fig. 1F and 1G). Pre-screening for the 8 most common karyotypic abnormalities in human iPSCs by hPSC Genetic Analysis (qPCR) indicated normal copy number ( $2.0 \pm 0.3$ ) at all tested loci (Fig. S1C). Karyostat analyses further verified both clones possess a normal female karyotype (46, XX) with no aneuploidies (Fig. 1H). Short tandem repeat (STR) typing was used to verify culture identity and purity; both iPSC clones matched the original parental lymphoblastoid cell line (data archived with journal). The original *ACTA1* mutation (c.553C > A) was confirmed to be present and heterozygous in both clones by PCR and Sanger sequencing (Fig. 1I). The iPSC lines were free of mycoplasma by a universal PCR test and agarose gel electrophoresis (Fig. S1C). Characterization of the iPSC clones is summarized in Table 2.

## 4. Materials and methods

### 4.1. Generation and maintenance of iPSC lines

Patient lymphoblastoid cell lines (LCLs) were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% L-glutamine (R10 medium) at 37 °C and 5% CO<sub>2</sub>. LCLs were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher). Briefly,  $3 \times 10^5$  cells were transduced at recommended multiplicity of infection and plated on growth factor-reduced (GFR) Matrigel® (1:100 in DMEM/F-12; ThermoFisher) in R10 medium. Cells were gradually adapted to mTESR™1 medium (StemCell) and individual clones picked for expansion and validation. iPSCs were cultured on GFR Matrigel and passaged every 3–5 days (at ~ 80% confluency) using 1X Versene (ThermoFisher). Cells were cryopreserved in 90% KnockOut™ Serum Replacement (ThermoFisher) with 10% DMSO. Control iPSCs were a gift from Prof. Rhonda Bassel-Duby and were maintained as above.

### 4.2. Immunocytochemistry – pluripotency marker expression and trilineage differentiation potential

For qualitative pluripotency analysis, iPSCs were plated on Matrigel-coated 96-well Nunc polymer optical bottom plates and stained for *OCT4*, *SSEA4*, *SOX2*, and *TRA-1-60* (Table 2). Trilineage differentiation potential was assessed using the STEMdiff™ Trilineage Differentiation Kit (StemCell) using StemPro™ Accutase™ (Gibco) for cell dissociation. Immunocytochemistry was performed as described in the PSC 4-marker Immunocytochemistry Kit (ThermoFisher), except that cells were incubated with primary antibodies (Table 3) overnight at 4 °C in 3% BSA. Nuclei were stained using NucBlue™ Fixed Cell stain (ThermoFisher).

**Table 1**  
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
HPIi001-A (iPS-6303-C6B3)	HPIi001-A	Female	4 months	Turkish	C/A	Nemaline myopathy 3; NEM3 (OMIM#161800), severe form
HPIi001-B (iPS-6303-R12)	HPIi001-B	Female	4 months	Turkish	C/A	Nemaline myopathy 3; NEM3 (OMIM#161800), severe form

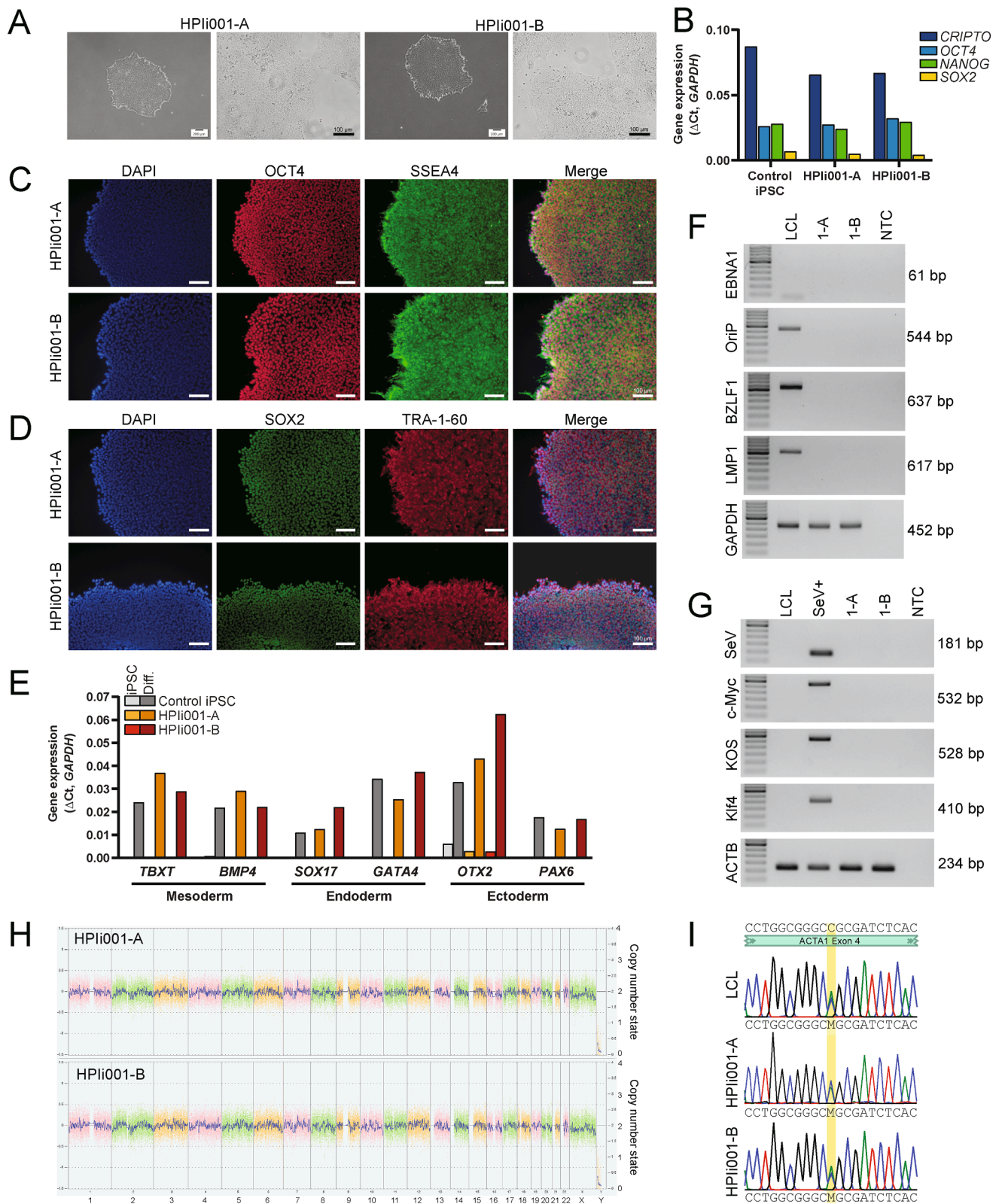


Fig. 1.

**Table 2**  
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography (light microscopy)	Normal	Fig. 1, panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for OCT4, SOX2, SSEA4, TRA-1-60	Fig. 1, panels C/D
Genotype	Quantitative analysis (qRT-PCR)	Expression of <i>OCT4</i> , <i>SOX2</i> , <i>NANOG</i> , <i>CRIPTO</i>	Fig. 1, panel B
Identity	KaryoStat™ assay (CytoScan Optima)	46, XX. No chromosomal aberrations were found in either line. Minimum resolution = 1 MB for losses, 2 MB for gains, 5 MB for LOH/AOH	Fig. 1, panel H
Mutation analysis (IF APPLICABLE)	Microsatellite PCR (mPCR) OR STR analysis	Microsatellite PCR not performed	Archived with journal
Microbiology and virology	Sanger sequencing	Matched to parental LCL line at 22/22 STR loci	Fig. 1, panel I
	Southern Blot OR WGS	Heterozygous <i>ACTA1</i> mutation; NM_001100:c.553C > A	
	Mycoplasma	Not performed	Supplementary Fig. 1, panel D
Differentiation potential	Directed differentiation, qPCR	Negative by PCR	Supplementary Fig. 1, panel E
	Directed differentiation, immunocytochemistry	Enrichment of <i>TBXT</i> and <i>BMP4</i> (mesoderm), <i>GATA4</i> and <i>SOX17</i> (ectoderm), and <i>OTX2</i> and <i>PAX6</i> (endoderm)	Supplementary Fig. 1, panel A/B
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Positive for OTX2 (ectoderm), Brachyury (mesoderm), <i>GATA4</i> (endoderm)	
Genotype additional info (OPTIONAL)	Blood group genotyping	Not performed	
	HLA tissue typing	Not performed	

Cells were imaged on an Olympus IX71 microscope with a DP74 camera and CellSens software.

#### 4.3. DNA and RNA extraction

Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN). RNA was extracted using the RNeasy Mini Kit (QIAGEN).

#### 4.4. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Total RNA was reverse transcribed into cDNA using the SuperScript™ III First-Strand Synthesis System (ThermoFisher). qRT-PCR was performed using the Rotor-Gene SYBR Green RT-PCR Kit on a Rotor-Gene Q thermocycler. Cycling conditions were as follows: 95 °C for 5 min, 45 cycles of 95 °C for 10 sec and 60 °C for 15 sec (acquiring), followed by melt curve analysis (60 °C to 95 °C, 1°/step). *GAPDH* was used for normalization, using the  $\Delta C_T$  method. Primers are listed in Table 3.

#### 4.5. Polymerase chain reaction (PCR)

EBV genes (*OriP*, *EBNA1*, *LMP1* and *BLZF1*) and a reference gene (*GAPDH*) were detected by PCR using GoTaq® G2 Hot Start Master Mix (Promega) with primers from Barrett et al., 2014. Presence of Sendai virus genome and transgenes were assessed by RT-PCR at passage 10 as per the CytoTune™ manufacturer's protocol. Transduced LCLs (day 3) were used as a positive control. Primers are listed in Table 3.

#### 4.6. KaryoStat analysis

Karyostat analysis was performed by the Ramaciotti Centre for Genomics (Sydney, NSW, Australia). Data were analyzed using Chromosome Analysis Suite 4.2 (ThermoFisher).

#### 4.7. Confirmation of pathogenic *ACTA1* variant

*ACTA1* exon 4 was amplified by PCR using GoTaq® G2 Hot Start Master Mix (Promega). Sanger sequencing was performed by the Australian Genome Research Facility (Perth, WA, Australia). Chromatograms were analyzed using Benchling (benchling.com). Primers are listed in Table 3.

#### 4.8. Mycoplasma testing

Lines were screened for mycoplasma using the ATCC Universal Mycoplasma PCR test kit.

#### 4.9. STR typing

STR typing was performed by PathWest Diagnostic Genomics (Perth, WA, Australia) using the QSTR Plus assay (Elucigene).

#### Declaration of Competing Interest

Nigel G Laing reports financial support was provided by A Foundation Building Strength (AFBS). Kristen J Nowak reports financial support was provided by The French Muscular Dystrophy Association (AFM-Telethon).

**Table 3**  
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Rabbit anti-OCT4	1:200	Thermo Fisher Scientific Cat# A24867, RRID: AB_2650999
Pluripotency marker	Mouse anti-SSEA4	1:100	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Pluripotency marker	Rat anti-SOX2	1:100	Thermo Fisher Scientific Cat# A24759, RRID: AB_2651000
Pluripotency marker	Mouse anti-TRA-1-60	1:100	Thermo Fisher Scientific Cat# A24868, RRID: AB_2651002
Secondary antibody	Alexa Fluor™ 594 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A21207, RRID: AB_141637
Secondary antibody	Alexa Fluor™ 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877, RRID: AB_2651008
Secondary antibody	Alexa Fluor™ 488 donkey anti-rat	1:250	Thermo Fisher Scientific Cat# A24876, RRID: AB_2651007
Secondary antibody	Alexa Fluor™ 594 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A21044, RRID: AB_2535713
Differentiation marker (ectoderm)	Anti-human Otx-2 NL557-conjugated goat IgG	1:10	R&D systems Cat# SC022, Part# 967389, RRID: Not in database
Differentiation marker (mesoderm)	Anti-human Brachyury NL557-conjugated goat IgG	1:10	R&D systems Cat# SC022, Part# 967388, RRID: Not in database
Differentiation marker (endoderm)	Anti-human GATA-4 NL493-conjugated goat IgG	1:10	R&D systems Cat# SC022, Part# 967391, RRID: Not in database
<b>Primers</b>			
	Target	Forward/Reverse primer (5'-3')	
Pluripotency markers (qPCR)	<i>OCT4</i>	F: GGGTTTTGGGATTAAGTTCTTCA R: GCCCCACCCTTTGTGTT	
	<i>SOX2</i>	F: CAAAAATGGCCATGCAGGTT R: AGTTGGGATCGAACAAAAGCTATT	
	<i>NANOG</i>	F: ACAACTGGCCGAAGAATAGCA R: GGTTCACAGTCGGGTTTCC	
Mesoderm markers (qPCR)	<i>CRIPTO</i>	F: CGGAACCTGTGAGCAGCATGT R: GGGCAGCCAGGTGTCATG	
	<i>TBXT</i>	F: GGTCCAGCCTTGGAAATGCCT R: CCGTTGCTCACAGACCACAG	
	<i>BMP4</i>	F: GCACTGGTCTTGGAGTATCCTG R: TGCTGAGGTTAAAGAGGAAACG	
Endoderm markers (qPCR)	<i>SOX17</i>	F: GTGGACCGCACGGAATTTGA R: GCTGTGCGGGAGATTACAC	
	<i>GATA4</i>	F: CAGCGAGGAGATGCGTCC R: AGACATCGCACTGACTGAGAA	
Ectoderm markers (qPCR)	<i>OTX2</i>	F: GACCCGGTACCCAGACATCTT R: GCGGCACTTAGCTCTTCGATT	
	<i>PAX6</i>	F: AACGATAACATACCAAGCGTGT R: GGTCTGCCCTTCAACATC	
House-keeping Genes (qPCR)	<i>GAPDH</i>	F: TCGGAGTCAACGGATTGGT R: TTGCCATGGGTGGAATCATA	
Sendai virus vectors (RT-PCR)	<i>SeV genome</i>	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAAGATATGTATC	
	<i>KOS transgene</i>	F: ATGCACCGCTACGACGTGAGCGC R: ACCTTGACAATCCTGATGTGG	
	<i>Klf4 transgene</i>	F: TTCCTGCATGCCAGAGGAGCCC R: AATGTATCGAAGGTGCTCAA	
	<i>c-Myc transgene BZLF-1</i>	F: TAACTGACTAGCAGGCTTGTCC R: TCCACATACAGTCTGGATGATGATG	

**Table 3 (continued)**

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
EBNA testing (PCR)			F: CACCTCAACCTGGAGACAAT R: TGAAGCAGGCGTGGTTTCAA <i>LMP1</i> F: ATGGAACACGACCTTGAGA R: TGAGCAGGATGAGGTCTAGG
	<i>EBNA1</i>		F: ATCAGGGCCAAGACATAGAGA R: GCCAATGCAACTTGGACGTT
	<i>OriP</i>		F: TCGGGGGTGTAGAGACAAC R: TTCCACGAGGGTAGTGAACC
House-keeping Genes (PCR)	<i>GAPDH</i>		F: ACCACAGTCCATGCCATCAC R: TCCACCACCCTGTGTCTGTA
Targeted mutation analysis (PCR/sequencing)	<i>ACTA1</i> exon 4 (F primer used for sequencing)		F: TAGCGCTGAGAGCCTAGCC R: CTGTGGTCCAGGAGGATAGC

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2021.102273>.

**References**

Barrett, R., Ornelas, L., Yeager, N., Mandefro, B., Sahabian, A., Lenaeus, L., Targan, S.R., Svendsen, C.N., Sareen, D., 2014. Reliable generation of induced pluripotent stem cells from human lymphoblastoid cell lines. *Stem Cells Transl. Med.* 3, 1429–1434.

Nowak, K.J., Wattanasirichaigoon, D., Goebel, H.H., Wilce, M., Pelin, K., Donner, K., Jacob, R.L., Hübner, C., Oexle, K., Anderson, J.R., Verity, C.M., North, K.N., Iannaccone, S.T., Müller, C.R., Nürnberg, P., Muntoni, F., Sewry, C., Hughes, I., Sutphen, R., Lacson, A.G., Swoboda, K.J., Vigneron, J., Wallgren-Pettersson, C., Beggs, A.H., Laing, N.G., 1999. Mutations in the skeletal muscle  $\alpha$ -actin gene in patients with actin myopathy and nemaline myopathy. *Nat. Genet.* 23 (2), 208–212.

Sanoudou, D., Beggs, A.H., 2001. Clinical and genetic heterogeneity in nemaline myopathy – a disease of skeletal muscle thin filaments. *Trends Mol. Med.* 7 (8), 362–368.

Sewry, C.A., Laitila, J.M., Wallgren-Pettersson, C., 2019. Nemaline myopathies: a current view. *J. Muscle Res. Cell Motil.* 40 (2), 111–126.

Sparrow, J.C., Nowak, K.J., Durling, H.J., Beggs, A.H., Wallgren-Pettersson, C., Romero, N., Nonaka, I., Laing, N.G., 2003. Muscle disease caused by mutations in the skeletal muscle alpha-actin gene (*ACTA1*). *Neuromuscul. Disord.* 13 (7-8), 519–531.