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Lab resource: Multiple cell lines

Generation of 5 induced pluripotent stem cell lines, LUMCi007-A and B and LUMCi008-A, B and C, from 2 patients with Huntington disease

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A B S T R A C T

Huntington disease (HD) is an autosomal dominant, neurodegenerative disease caused by a CAG repeat expansion within the coding sequence of the *HTT* gene, resulting in a highly toxic protein with an expanded polyglutamine stretch that forms typical protein aggregates throughout the brain. We generated human induced pluripotent stem cells (hiPSCs) from two HD patients using non-integrating Sendai virus (SeV). The hiPSCs display a normal karyotype, express all pluripotency markers, have the same CAG repeat expansion as the original fibroblasts and are able to differentiate into the three germ layers *in vitro*.

1. Resource utility

The newly generated hiPSCs are useful to study HD disease mechanisms. Furthermore, these cells can be used for disease modelling and drug discovery studies.

2. Resource details

Huntington Disease (HD) is an autosomal dominant, neurodegenerative disease, clinically characterized by motor disturbances, especially chorea (*i.e.* involuntary and unpredictable body movements), behavioral and psychiatric symptoms and cognitive decline (Roos, 2010). As the disease progresses, degeneration can be seen throughout the brain, but the main affected brain regions are the striatum and cerebral cortex (Gusella et al., 1983). HD is caused by a CAG repeat expansion in the first exon of the huntingtin (*HTT*) gene on chromosome 4p16.3. The *HTT* RNA is translated into a highly toxic huntingtin protein with an expanded stretch of glutamine amino acids that aggregates throughout the brain.

Skin biopsies were obtained from two symptomatic HD patients; a 51 year old male (CAG repeat 18/46, age of onset 47) and a 49 year old female (CAG repeat 19/46, age of onset 47). The obtained skin fibroblasts were succesfully reprogrammed into hiPSCs using a replication-defective and persistent Sendai virus (SeV) vector installed with OCT4, SOX2, KLF4 and c-Myc (Nishimura et al., 2011). The hiPSC clones derived from the male were named LUMCi007-A and B and the hiPSCs derived from the female were named LUMCi008-A, B and C (Table 1). All hiPSC clones showed typical hiPSC-like morphology with small and tightly packed cells, a high nucleus to cytoplasm ratio and well defined nucleoli (Fig. 1A) and were SeV negative at passage 5 as shown by immunofluorescent staining (Supplementary Fig. S1A) and RT-qPCR (data not shown). All clones stained positive for pluripotency markers OCT3/4, NANOG and SSEA-4 (Fig. 1B). Accordingly, the hiPSCs showed, compared to the original fibroblasts, upregulated expression of pluripotency genes OCT3/4, NANOG and SOX2 (Fig. 1C). A routine Global Screening Array (GSA) showed no major allelic changes in the hiPSC clones, tested at passage 5–6 (Fig. 1D). Furthermore, the newly generated hiPSCs were identical to their original fibroblasts as shown by comparison of the allelic calls (Supplementary Fig. S1B). The CAG repeat size of the new hiPSC lines was confirmed by PCR (Fig. 1E) and fragment analysis (data not shown). Trilineage *in vitro* differentiation of all clones led to expression of the endodermal marker Sry-related HMG

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Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus (#CAG repeats)	Disease
LUMCi007-A	151–1	Male	51	Caucasian	18/46	Huntington disease
LUMCi007-B	151–5	Male	51	Caucasian	18/46	Huntington disease
LUMCi008-A	152–2	Female	49	Caucasian	19/46	Huntington disease
LUMCi008-B	152–3	Female	49	Caucasian	19/46	Huntington disease
LUMCi008-C	152–4	Female	49	Caucasian	19/46	Huntington disease

box 17 (SOX17), the mesodermal marker smooth muscle actin (SMA), the ectodermal marker β3-tubulin (TUBB3) as confirmed by immunofluorescent staining (Fig. 1F). Finally, all cells tested negative for mycoplasma (Supplementary Fig. S1C). All the above mentioned data are presented in Table 2.

3. Materials and methods

3.1. Ethical statement

This study was approved by the LUMC scientific ethical committee and informed consent was obtained from the two HD patients (NL45478.058.13/P13.080).

3.2. Generation of hiPSCs

A skin biopsy was obtained from a 51 year old male and a 49 year old female. Both with symptomatic HD. After dissection, fibroblasts were cultured in DMEM/F12 medium containing Glutamax, 10% Foetal Bovine Serum (FBS), Non-Essential Amino Acids (NEAA), 2-mercaptoethanol, Pen/Strep (all Gibco) and fresh 0.01 mg/ml vitamin C (Sigma) at 37 °C and 5% CO_2. Fibroblasts were expanded to passage 3 and subsequently used for reprogramming. For this, 1×10^5 fibroblasts were transduced with 7.5 MOI SeVdp(KOSM)302 L (Nishimura et al., 2011) and seeded on irradiated mouse embryonic fibroblasts (MEFs) in fibroblast medium. The next day, medium was changed to DMEM/F12 medium containing Glutamax, 20% KnockOut Serum Replacement (KOSR), Non-Essential Amino Acids (NEAA), 2-mercaptoethanol, Pen/Strep (Gibco) and 10 ng/ml bFGF (Peprotech) until hiPSC colonies formed after approximately 3 weeks. hiPSC colonies were excised manually and expanded on Vitronectin XF coated plates in TESR-E8 medium (STEMCELL Technologies), resulting in 2 and 3 clones of both patient cell lines. iPSCs were further cultured on Matrigel (Corning) coated plates, using mTeSR1 medium (STEMCELL Technologies). Passaging was performed using a 5 min incubation with ReLeSR (STEMCELL Technologies) at 37 °C. iPSCs were passaged 1:10–1:20 every 5–7 days and cultured at 37 °C in 5% CO_2.

3.3. Trilineage differentiation in vitro of hiPSCs

After incubation with Gentle Cell Dissociation Reagent (STEMCELL Technologies), undifferentiated hiPSC colonies were harvested and plated on Matrigel (Corning) coated glass coverslips according to the STEMdiff™ Trilineage Differentiation Kit protocol (STEMCELL Technologies). Medium was changed daily and hiPSCs were fixed with 2% paraformaldehyde (PFA) for immunofluorescent stainings after 5 (endoderm and mesoderm) and 7 days (ectoderm).

3.4. Immunofluorescent staining

After fixation in 2% PFA for 30 min at RT, the hiPSCs were permeabilized with 0.1% Triton X-100 for 1 h at RT. Next, the cells were incubated with blocking buffer (4% normal swine serum (NSS, DAKO)) for 1 h at RT. Primary antibody labeling, diluted in 4% NSS, was performed O/N at 4 °C. After washing, the cells were incubated with secondary fluorescent antibodies for 1 h at RT. DAPI was used as a nuclear staining. Antibodies are listed in Table 3. Images were obtained on a Leica TCS SP8 microscope.

3.5. RNA isolation and RT-qPCR

RNA isolation was performed using the ReliaPrep™ Miniprep System (Promega). In short, 500 ng RNA was used per reaction for cDNA synthesis, using the transcriptor first strand cDNA synthesis kit (Roche). RT-qPCR reactions were run, in 3 biological and 3 technical triplicates, on a LightCycler© 480 Real-Time PCR System (Roche), using SensiMix SYBR Hi-ROX kit (Bioline). Cycle parameters were: 10 min at 95 °C and 45 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 20 s. CT-values were normalized to GAPDH, using the ΔΔCT-method. HiPSC line 114–2 was used as a positive control. As a negative control, fibroblast line 2B was used (Buijsen et al., 2018). Primer sequences are listed in Table 3.

3.6. Genomic DNA isolation

Genomic DNA isolation was performed using the Wizard Genomic DNA Purification Kit (Promega), according to manufacturer's instructions.

3.7. Repeat length PCR

The CAG repeat in the first exon of the *HTT* gene was amplified to confirm the genotype of the hiPSCs. Cycle parameters were: 4 min at 95 °C and 35 cycles of 30 s at 95 °C, 30 s at 59 °C and 70 s at 72 °C, ended by 7 min at 72 °C. The PCR products were run on an ABI 3730 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA). As a control, hiPSC line 114–2 was used (Buijsen et al., 2018). Primers are listed in Table 3.

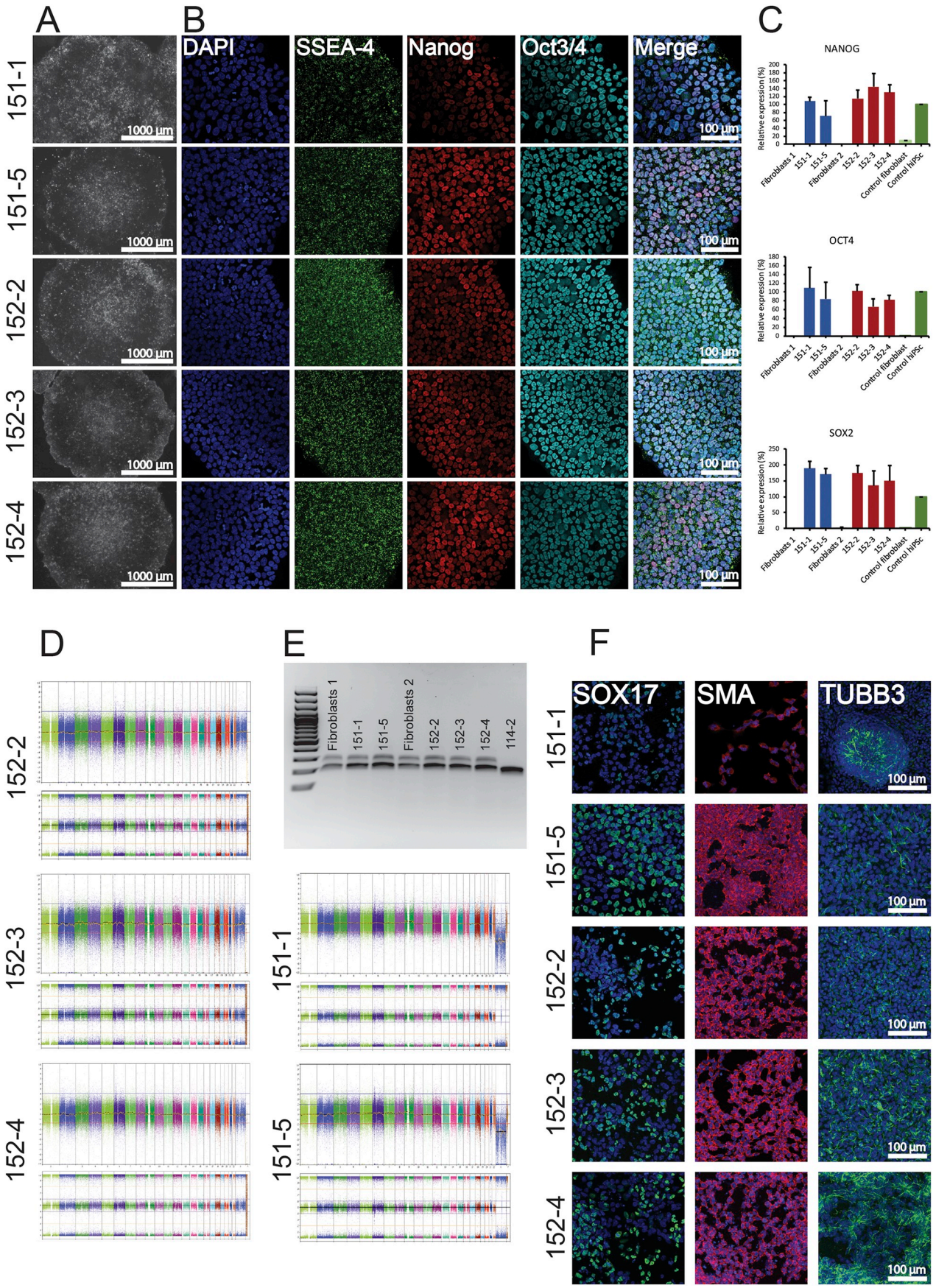
A
B
C
DAPI
SSEA-4
Nanog
Oct3/4
Merge
151-1
151-5
152-2
152-3
152-4
1000 µm
100 µm
NANOG
OCT4
SOX2
Relative expression (%)
Fibroblasts 1
151-1
151-5
Fibroblasts 2
152-2
152-3
152-4
Control fibroblast
Control hiPSc
D
E
F
114-2
SOX17
SMA
TUBB3

Fig. 1.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Brightfield microscopy	Normal morphology	Fig. 1, panel A
Phenotype	Qualitative analysis of immunofluorescent staining	Expression of pluripotency markers NANOG, SSEA-4, Oct-3/4	Fig. 1, panel B
	Quantitative analysis by RT-qPCR	Expression of pluripotency markers NANOG, SOX2, Oct-3/4	Fig. 1, panel C
Genotype	GSAMD24 v2 Illumina Infinium SNP array 760 k	CNV report resolution 50 kb: No major copy number variations or allelic changes	Fig. 1, panel D
Identity	GSAMD24 v2 Illumina Infinium SNP array 760 k	Fibroblasts and hiPSCs have > 99,99% identical SNPs	Summarized data in figure S1, panel B. Raw data available with the authors
Mutation analysis	Repeat length PCR and fragment analysis	Fibroblasts and derived hiPSCs have identical CAG repeat lengths	Fig. 1, panel E (PCR) and data not shown, but available from author (fragment analysis)
	Southern Blot OR WGS	N/A	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: negative	Fig. S1, panel C.
Differentiation potential	Qualitative analysis of immunofluorescent staining	Positive staining of germ layer markers SOX17 (endoderm), SMA (mesoderm) and TUBB3 (ectoderm)	Fig. 1, panel F
Donor screening	N/A		
Genotype additional info	Blood group genotyping	N/A	
	HLA tissue typing	N/A	

3.8. Fragment length analysis

Fragment length analysis was performed with OneTaq Master Mix (New England Biolabs) on an ABI genetic analyser (ThermoFisher). Cycle parameters were: 5 min at 94 °C and 35 cycles of 30 s 94 °C, 1 min 60 °C and 2 min 68 °C. Primers are listed in Table 3.

3.9. Mycoplasma detection

To test the presence of mycoplasma, the MycoAlert™ Mycoplasma Detection Kit (Lonza) was used, according to manufacturer's instructions.

3.10. Global screening array

A Global screening array (GSA) (Illumina) was used according to standard protocols, followed by an analysis in GenomeStudio software (Illumina). The GenomeStudio Final reports were used to analyze and visualize the results in Nexus Discovery (BioDiscovery El Segundo). A report resolution of 50 kb was used to analyze the data for chromosomal aberrations. To compare the patient fibroblasts with the generated hiPSCs, the array final reports were selected as input for an R script. Using statistics we determined whether the allelic calls of the fibroblasts and hiPSCs matched, mismatched or failed.

3.11. Key resources table

Unique stem cell lines identifier	LUMCi007-A LUMCi007-B LUMCi008-A LUMCi008-B LUMCi008-C
Alternative names of stem cell lines	LUMCi007-A: LUMC0151iHD01 and 151–1 LUMCi007-B: LUMC0151iHD05 and 151–5 LUMCi008-A: LUMC0152iHD02 and 152–2 LUMCi008-B: LUMC0152iHD03 and 152–3 LUMCi008-C: LUMC0152iHD04 and 152–4
Institution	Leiden University Medical Center (LUMC), Leiden, The Netherlands
Contact information of distributor	Dr. Willeke M.C. van Roon-Mom, W.M.C.van_Roon@lumc.nl
Type of cell lines	hiPSC
Origin	Human
Cell source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Non-integrating Sendai virus
Multiline rationale	2 disease cell lines (2 and 3 clones per line)
Gene modification	Yes
Type of modification	Hereditary
Associated disease	Huntington disease
Gene/locus	*HTT*/4p16.3
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	June 2018
Cell line repository/bank	https://hpscreg.eu/cell-line/LUMCi007-A https://hpscreg.eu/cell-line/LUMCi007-B https://hpscreg.eu/cell-line/LUMCi008-A https://hpscreg.eu/cell-line/LUMCi008-B https://hpscreg.eu/cell-line/LUMCi008-C
Ethical approval	NL45478.058.13/P13.080, Medical Ethical Committee (MEC), Leiden University Medical Center (LUMC). Informed consent was obtained from both HD patients.

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse IgG2b anti-Oct-3/4	1:100	Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051
	Mouse IgG1 anti-Nanog	1:150	Santa Cruz Biotechnology Cat# sc-293,121, RRID:AB_2665475
	Mouse IgG3 anti-SSEA-4	1:30	BioLegend Cat# 330402, RRID:AB_1089208
Differentiation markers	Goat IgG anti-SOX17	1:100	R and D Systems Cat# AF1924, RRID:AB_355060
	Mouse IgG2a anti-SMA	1:3000	Sigma-Aldrich Cat# A2547, RRID:AB_476701
	Mouse IgG2a anti-β3-tubulin	1:4000	Covance Research Products Inc. Cat# MMS-435P, RRID:AB_2313773
Secondary antibodies	Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	1:250	Thermo Fisher Scientific Cat# A-21242, RRID:AB_2535811
	Goat anti-Mouse IgG3 Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:250	Thermo Fisher Scientific Cat# A-21151, RRID:AB_2535784
	Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:250	Thermo Fisher Scientific Cat# A-21124, RRID:AB_2535766
	Goat anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A-11031, RRID:AB_144696

Primers		
	Target	Forward/Reverse primer (5′-3′)
SeV based vectors (qPCR)	SeV	GCAGCTCTAACGTTGTCAAA/CCTGGAGCAAATTCACCATGA
	GAPDH	TCCTCTGACTTCAACAGCGA/GGGTCTTACTCCTTGGAGGC
Pluripotency markers (qPCR)	NANOG	CAGTCTGGACACTGGCTGAA/CTCGCTGATTAGGCTCCAAC
	OCT4	TGTACTCCTCGGTCCCTTTC/TCCAGGTTTTCTTTCCTAGC
	SOX2	GCTAGTCTCCAAGCGACGAA/GCAAGAAGCCTCTCCTTGAA
House-keeping genes (qPCR)	GAPDH	AGCCACATCGCTCAGACACC/GTACTCAGCGGCCAGCATCG
Genotyping by repeat length PCR	HTT	ATGGCGACCCTGGAAAAGCTGAT/TGAGGCAGCAGCGGCTG
Targeted mutation analysis (Fragment length analysis)	HTT	ATGAAGGCCTTCGAGTCCCTCAAGTCCTTC/GGCGGTGGCGGCTGTTGCTGCTGC

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Declaration of Competing Interests

The authors declare to have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2019.101498.

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