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# Generation of spinocerebellar ataxia type 2 patient-derived iPSC line H266

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ABSTRACT

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease primarily affecting the cerebellum. Very little is known about the molecular mechanisms underlying the disease and, to date, no cure or treatment is available. Here, we demonstrate the generation of an induced pluripotent stem cell (iPSC) line of a SCA2 patient. The selected clone has been proven to be a *bona fide* iPSC line, which retains a normal karyotype. Due to its differentiation potential into neurons, this iPSC line will be a valuable tool in studying a disease-specific phenotype of SCA2.



## 1. Resource table

| | |
|---|---|
| Name of Stem Cell construct | H266 clone10 |
| Institution | University of Copenhagen and Bioneer A/S |
| Person who created resource | Adele G. Marthaler, Benjamin Schmid |
| Contact person and email | Adele G. Marthaler, adele.marthaler@sund.ku.dk |
| Date archived/stock date | July 2014 |
| Origin | Human skin fibroblasts |
| Type of resource | Induced pluripotent stem cells; derived from skin fibroblasts of patient with spinocerebellar ataxia type 2 |
| Sub-type | Cell line |
| Key transcription factors | Episomal plasmids containing *hOCT4*, *hSOX2*, *hL-MYC*, *hKLF4*, *hLIN28*, and *shP53* (Addgene plasmids 27077, 27078 and 27080; Okita et al., 2011) |
| Authentication | Identity and purity of stem cell line confirmed (Fig. 1) |
| Link to related literature (direct URL links and full references) | |
| Information in public databases | |

## 2. Resource details

Human skin fibroblasts, obtained by skin biopsy of a symptomatic, female 25-year-old spinocerebellar type 2 (SCA2) patient (anonymized as H266), were reprogrammed using episomal vectors carrying transcripts for human *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, and small hairpin RNA for *TP53* (Okita et al., 2011). The clone described in this publication was termed H266 clone (c) 10. The absence of the reprogramming plasmids was confirmed by quantitative PCR (qPCR) on genomic DNA (Fig. 1A).

SCA2 is a dominantly inherited neurodegenerative disorder caused by a mutation in the *ATXN2* gene. Normal alleles contain 22 CAG repeats with CAA interruptions (also coding for glutamine), whereas disease causing alleles contain trinucleotide repeats of 33 or more CAGs (usually without any CAA interruptions) (Pulst et al., 1996). The repeat lengths for patient H266 were determined to be 22 and 44 by fragment length analysis (data not shown) and confirmed to be present also in iPSC line H266 c10 by sequencing both alleles individually (Fig. 1B).

Furthermore, the expression of key pluripotency genes was observed both on RNA, as well as protein level, as demonstrated by qRTPCR analysis and immunocytochemistry, respectively (Fig. 1C and D). Additionally, the cells had the capacity to form derivatives of all three germ layers upon embryoid body differentiation (Fig. 1E). Taken together, this validates the true pluripotent potential of the generated iPCS line.

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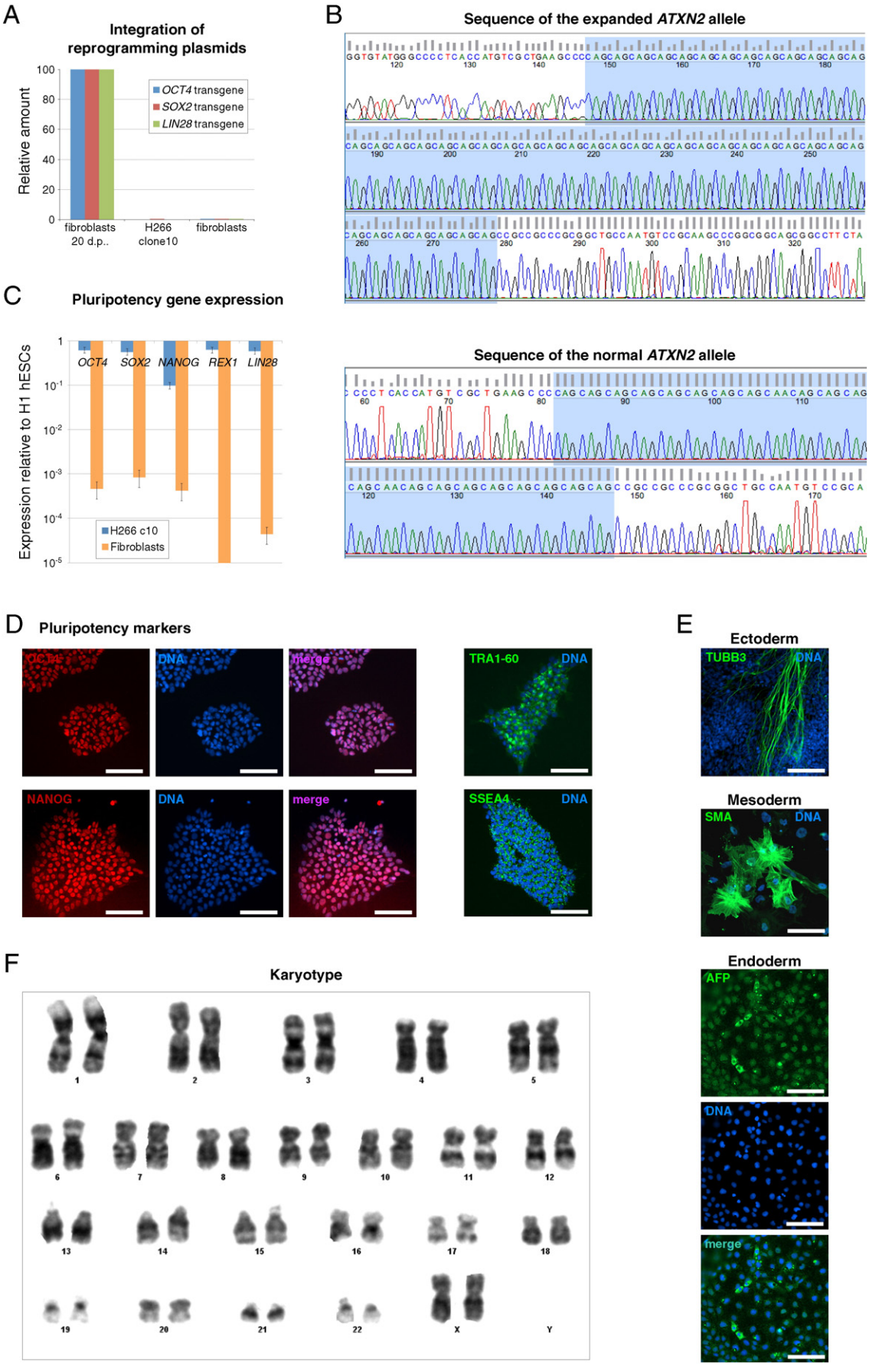


**Fig. 1.** (*caption on page 168*).

## 3. Materials and methods

### 3.1. Reprogramming using episomal vectors

Fibroblasts were cultured in fibroblast medium, consisting of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin and streptomycin (Pen/Strep). $1 \times 10^5$ fibroblasts were electroporated with a total of 1 μg of episomal plasmids containing *hOCT4* with a short hairpin to *TP53* (*shP53*; Addgene plasmids 27077 and 27076, respectively), *hSOX2* and *hKLF4* (Addgene plasmid 27078), and *hL-MYC* and *hLIN28* (Addgene plasmid 27080), (Okita et al., 2011), and cultured in fibroblast medium. Electroporation was carried out using a Neon™ electroporation device with two pulses at 1200 V for 20 ms (Life Technologies). One week after electroporation, fibroblasts were trypsinized and split 1:2 onto matrigel-coated dishes (BD Biosciences) and cultured in E8 medium (Gibco) under hypoxic conditions. After four weeks, primary iPSC colonies were picked to establish stable clones.

### 3.2. Integration of episomal vectors

DNA for integration analysis was purified from fibroblasts and iPSCs using the DNeasy Blood and Tissue kit (Qiagen). Data was plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$ with fibroblasts and *GAPDH* as references. The following primers were used:

| | |
|---|---|
| *OCT4* transgene for | 5′-CATTCAAACTGAGGTAAGGG-3′ |
| *OCT4* transgene rev | 5′-TAGCGTAAAAGGAGCAACATAG-3′ |
| *SOX2* transgene for | 5′-TTCACATGTCCCAGCACTACCAGA-3′ |
| *SOX2* transgene rev | 5′-TTTGTTTGACAGGAGCGACAAT-3′ |
| *LIN28* transgene for | 5′-AGCCATATGGTAGCCTCATGTCCGC-3′ |
| *LIN28* transgene rev | 5′-TAGCGTAAAAGGAGCAACATAG-3′ |

### 3.3. Sequencing

Sanger sequencing of a 300 base pair region around the CAG repeat region of the *ATXN2* gene was carried out in an ABI PRISM 310 Genetic Analyzer using the primers SCA2 seq2 forward 5′-CTTGGTCTCGGCGG GC-3′ and SCA2 seq2 reverse 5′-GAGGAGACCGAGGACGAGG-3′.

### 3.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

RNA was extracted using RNeasy Mini (QIAGEN) and cDNA synthesis was performed using Revert Aid First Strand cDNA synthesis kit (Thermo Scientific) according to the manufacturers' protocols. qRT-PCR was carried out using LightCycler 480 SYBR Green I Master (Roche). Data was plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$. The following primers were used:

| | |
|---|---|
| *OCT4* for | 5′-CCCCAGGGCCCCATTTTGGTACC-3′ |
| *OCT4* rev | 5′-ACCTCAGTTTGAATGCATGGGAGAGC-3′ |
| *SOX2* for | 5′-TTCACATGTCCAGCACTACCAGA-3′ |
| *SOX2* rev | 5′-TCACATGTGTGAGAGGGGCAGTGTGC-3′ |
| *NANOG* for | 5′-AAAGAATCTTCACCTATGCC-3′ |
| *NANOG* rev | 5′-GAAGGAAGAGGAGAGACAGT-3′ |
| *REX1* for | 5′-TTTCTGAGTACGTGCCCAGGCAA-3′ |
| *REX1* rev | 5′-CTCTGAGAAAGCATCTCTCCTTC-3′ |
| *LIN28* for | 5′-AGCCATATGGTAGCCTCATGTCCGC-3′ |
| *LIN28* rev | 5′-TCAATTCTGTGCCTCCGGGAGCAGGGTAGG-3′ |
| *ACTB* for | 5′-TCAAGATCATTGCTCCTCCTGAG-3′ |
| *ACTB* rev | 5′-ACATCTGCTGGAAGGTGGACA-3′ |
| *RPL13A* for | 5′-TTCCAAGCGGCTGCCGAAGA-3′ |
| *RPL13A* rev | 5′-TTCCGGCCCAGCAGTACCTGT-3′ |
| *HSP90AB1* for | 5′-TCCGGCGCAGTGTTGGGAC-3′ |
| *HSP90AB1* rev | 5′-TCCATGGTGCACTTCCTCAGGC-3′ |
| *GUSB* for | 5′-TCCGCGCCGACTTCTCTGACA-3′ |
| *GUSB* rev | 5′-AAATGCCGCAGACGCCAGTCC-3′ |

### 3.5. Immunocytochemistry

Immunocytochemistry was performed as previously described (Marthaler et al., 2013). The following primary antibodies were used: Anti-OCT4 (Santa Cruz, sc 8628); anti-NANOG (Peprotech, 500P236); anti-TRA1-60 (BioLegend, 330602); anti-SSEA4 (BioLegend, 330402); anti-TUBB3 (Millipore, MAB1637); anti-SMA (Dako, M0851), anti-AFP (Dako, A0008); all 1:500. Secondary antibodies used were: Alexa Fluor 594 rabbit anti-goat (A21223) and goat anti-rabbit (A11072), Alexa Fluor 488 donkey anti-rabbit (A21206), donkey anti-goat (A11055), and goat anti-mouse (A11017), all 1:2000, (Invitrogen).

### 3.6. Embryoid body differentiation

iPSCs growing in E8 medium (Gibco) on matrigel (Corning Bioscience) were dissociated with EDTA (Gibco) and allowed to form aggregates in none-coated cell culture dishes. On day 3, aggregates were transferred to matrigel-coated dishes and medium was switched to differentiation medium: DMEM/F12 containing 20% FBS, L-glutamine, and non-essential amino acids (all Gibco) for meso- and endoderm induction, or DMEM/F12 containing 50% neurobasal medium, B27, N2, and L-glutamine (all Gibco) for ectoderm induction. Cells were fixed for immunocytochemistry on day 14.

### 3.7. Verification and authentication

An intact genome with no detectable abnormalities was demonstrated by karyotyping using G-banding (Fig. 1F). Analysis was performed by Cell Guidance Systems, Cambridge, UK.

### 3.8. Ethical approval

The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (RH), (protocol number H-4-2011-157), Copenhagen, Denmark, and written informed consent was obtained in all cases.

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**Fig. 1.** H266 c10 is a *bona fide* iPSC line with a normal karyotype. (A) The absence of reprogramming plasmids was confirmed by quantitative PCR on genomic DNA of H266 c10. Fibroblasts 20 days post-electroporation or non-electroporated fibroblasts served as positive and negative control, respectively. Data was normalized to *GAPDH* and plotted using the delta delta Ct algorithm, $2^{(-\Delta\Delta Ct)}$. Due to location on the same plasmid, *shp53*, *KLF4*, and *L-Myc* are represented by *OCT4*, *SOX2*, and *LIN28*, respectively. (B) Sequencing of the expanded and the normal *ATXN2* allele showed 44 and 22 trinucleotide repeats, respectively, highlighted in blue. (C) Expression levels of pluripotency genes measured by qRT-PCR. Data were plotted relative to H1 human ESCs. Fibroblasts served as a negative control. Error bars indicate standard error of calculations based on ΔΔCt-values obtained from four housekeeping genes, *ACTB*, *RPL37a*, *HSP90AB1*, and *GUSB*. (D) OCT4, NANOG, TRA1-60, and SSEA4 immunofluorescence images of H266 c10, counterstained with Hoechst. Scale bars, 100 μm. (E) Immunocytochemistry for marker proteins representative of the three germ layers, TUBB3 (ectoderm), SMA (mesoderm), and AFP (endoderm), after *in vitro* differentiation of H266 c10 by embryoid body formation. Scale bars, 100 μm. (F) Chromosome analysis of H266 c10 showing a normal 46, XX karyotype.

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