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Lab resource: Stem cell line

Generation of an isogenic, gene-corrected control cell line of the 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. We have successfully generated *bona fide* induced pluripotent stem cell (iPSC) lines of SCA2 patients in order to study a disease-specific phenotype. Here, we demonstrate the gene correction of the iPSC line H266 clone 10 where we have exchanged the expanded CAG repeat of the *ATXN2* gene with the normal length found in healthy alleles. This gene corrected cell line will provide the ideal control to model SCA2 by iPSC technology.

1. Resource table

Name of stem cell construct	H266 clone10 GC
Institution	University of Copenhagen and Bioneer A/S
Person who created resource	Adele G. Marthaler, Alisa Tubsuwan
Contact person and email	Adele G. Marthaler, adele.marthaler@sund.ku.dk
Date archived/stock date	May 2015
Origin	Human induced pluripotent stem cell line H266 clone 10
Type of resource	Gene-corrected induced pluripotent stem cells; originally 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

An induced pluripotent stem cell (iPSC) line had been generated from human skin fibroblasts of a female, symptomatic 25-year-old spinocerebellar type 2 (SCA2) patient (anonymized as H266) using episomal vectors carrying transcripts for human *OCT4*, *SOX2*, *KLF4*, *L-MYC*, *LIN28*, and small hairpin RNA for *TP53* (Okita et al., 2011). This cell line, H266 clone (c) 10, has been described as a *bona fide* iPSC line with a normal karyotype (Marthaler et al., 2013-in this issue).

We have generated a gene-corrected clone of H266 c10 using the CRISPRs/Cas9 system (Ran et al., 2013), where the expanded 44 CAG region in the *ATXN2* gene has been replaced with a wildtype 22 CAG repeat (Fig. 1A). Successful exchange was validated by sequencing (Fig. 1B). We have furthermore confirmed that the DNA sequence stayed intact and no frameshift or other mutation had been introduced into the gene edited site, by analyzing the region around the CRISPR cutting site (nucleotides 121–143 in Fig. 1A).

Subsequently, we confirmed that the gene corrected clone of H266 c10, termed H266 c10 GC, remained truly pluripotent. This was demonstrated by expression of key pluripotency markers on RNA, as well as protein level (Fig. 1 C and D). Additionally, H266 c10 GC retained the potential to differentiate into cell types of the three germ layers upon embryoid body formation (Fig. 1E). More importantly, no genetic chromosomal aberrations were introduced by the gene editing process and the cells still exhibit a normal karyotype (Fig. 1F).

In summary, we have generated an isogenic, gene-corrected iPSC line of an existing SCA2 iPSC line. Together with two more SCA2

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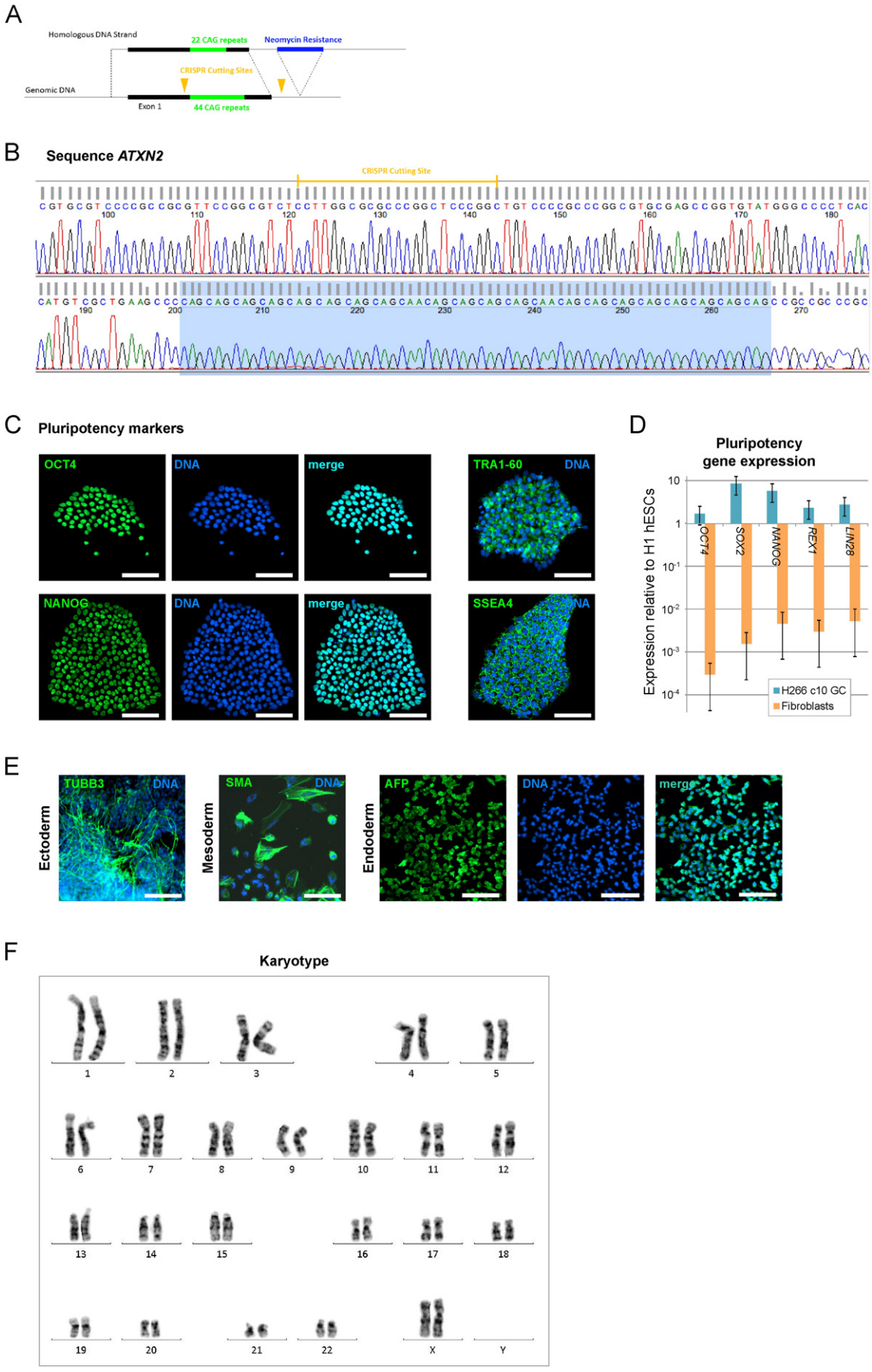
A
Homologous DNA Strand
22 CAG repeats
Neomycin Resistance
CRISPR Cutting Sites
Genomic DNA
Exon 1
44 CAG repeats
B
Sequence ATXN2
CRISPR Cutting Site
C
Pluripotency markers
OCT4
DNA
merge
TRA1-60
DNA
NANOG
DNA
merge
SSEA4
DNA
D
Pluripotency gene expression
Expression relative to H1 hESCs
OCT4
SOX2
NANOG
REX1
LIN28
H266 c10 GC
Fibroblasts
E
Ectoderm
TUBB3
DNA
Mesoderm
SMA
DNA
Endoderm
AFP
DNA
merge
F
Karyotype
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