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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 H196

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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 H196 clone 7 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.

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## 1. Resource table

| Name of stem cell construct | H196 clone7 GC |
|---|---|
| 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 2015 |
| Origin | Human induced pluripotent stem cell line H196 clone 7 |
| 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 pluripoten stem cell (iPSC) line had been generated from human skin fibroblasts of a male, symptomatic 52-year-old spinocerebellar type 2 (SCA2) patient (anonymized as H196) 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, H196 clone (c) 7, has been described as a *bona fide* iPSC line with a normal karyotype (Marthaler et al., submitted to Stem Cell Research).

We have generated a gene-corrected clone of H196 c7 using the CRISPRs/Cas9 system (Ran et al. 2013), where the expanded 36 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 (nucleotide 119–141 in Fig. 1A).

Subsequently, we confirmed that the gene corrected clone of H196 c7, termed H196 c7 GC, remained truly pluripotent. This was demonstrated by expression of key pluripotency markers on RNA, as well as protein level (Fig. 1C and D ). Additionally, H196 c7 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).

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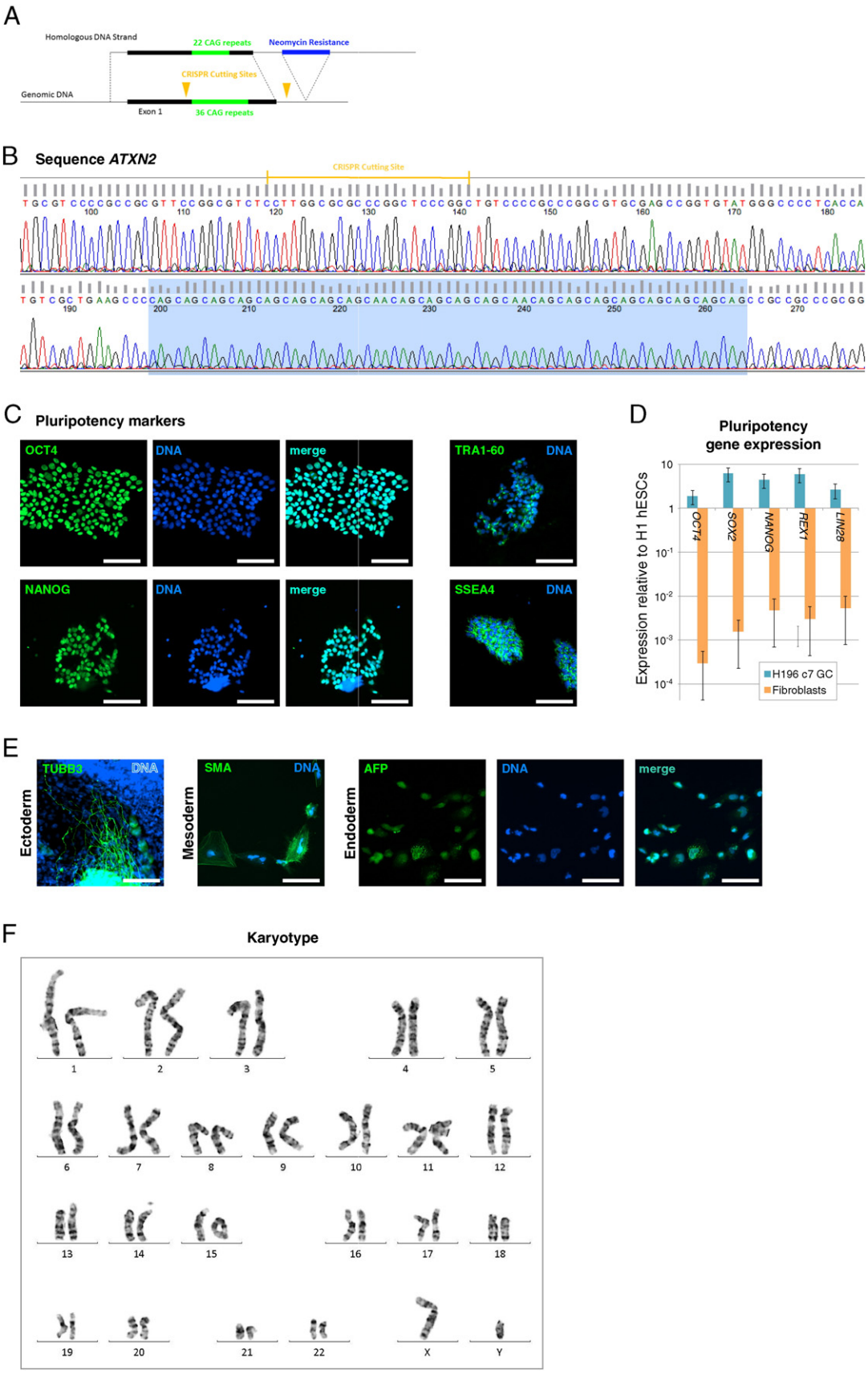


**Fig. 1.** (*caption on page 164*).

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