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Lab resource: Stem cell line

Induced pluripotent stem cells (iPSCs) derived from a pre-symptomatic carrier of a R406W mutation in microtubule-associated protein tau (MAPT) causing frontotemporal dementia

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ABSTRACT

Skin fibroblasts were obtained from a 28-year-old pre-symptomatic woman carrying a R406W mutation in microtubule-associated protein tau (MAPT), known to cause frontotemporal dementia. Induced pluripotent stem cell (iPSCs) were established by electroporation with episomal plasmids containing *hOCT4*, *hSOX2*, *hKLF2*, *hL-MYC*, *hLIN-28* and *shP53*. iPSCs were free of genomically integrated reprogramming genes, contained the expected c.1216C>T substitution in exon 13 of the *MAPT* gene, expressed the expected pluripotency markers, displayed in vitro differentiation potential to the three germ layers and had normal karyotype. The iPSC line may be useful for studying hereditary frontotemporal dementia and TAU pathology in vitro.

Resource table:

Name of stem cell construct	H236 C6
Institution	Bioneer A/S
Person who created resource	Mikkel Aabech Rasmussen, Bjørn Holst
Contact person and email	Bjørn Holst, bho@bioneer.dk
Date archived/stock date	July 1, 2012
Origin	Human skin fibroblasts
Type of resource	Biological reagent: induced pluripotent stem cell (iPS); derived from a MAPT R406W mutation carrier
Sub-type	Induced pluripotent stem cell
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 cell line confirmed by integration analysis, sequencing of mutation, pluripotency analysis, karyotyping and in vitro differentiation (Fig. 1).
Link to related literature (direct URL links and full references)	http://onlinelibrary.wiley.com/doi/10.1111/j.1468-1331.2008.02069.x/abstract;jsessionid=175DB65708CDD60137A0E1D39E93D3D6.f04t01 The MAPT R406W carrier is a pre-symptomatic daughter of a patient diagnosed with frontotemporal dementia which is also heterozygous for the MAPT R406W mutation http://www.sciencedirect.com/science/article/pii/S1873506115001865
Information in public databases	Link to any data or information about this resource in a database if applicable

1. Resource details

Fibroblasts were obtained from a 28-year old woman heterozygous for a R406W mutation in microtubule-associated protein tau (MAPT), which can cause frontotemporal dementia. However, the woman was pre-symptomatic at the time of biopsy (Lindquist et al., 2008). Reprogramming was performed by electroporation with three episomal plasmids containing *hOCT4* with or without a short hairpin to TP53 (*shp53*), *hSOX2* and *hKLF4*, and *hL-MYC* and

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hLIN28 (Okita et al., 2011). This method had previously been used to establish integration-free iPSC from an 18-year old healthy male (Rasmussen et al., 2014). Four weeks after reprogramming, an average of 32 colonies per 1×10^5 fibroblasts (0.03%) emerged with the inclusion of *shp53*, whereas, no colonies were observed without *shp53*. Integration analysis with plasmid-specific primers showed that *hOCT4*, *hSOX2* and *hLIN28*, present on each of the three plasmids, had not integrated into the genome (Fig 1A) and sequencing confirmed the presence of a c.1216C>T substitution in one of the alleles of exon 13 in the *MAPT* gene corresponding to a R406W mutation (Fig 1B). Pluripotency analysis showed that transcription from the endogenous pluripotency genes *NANOG*, *POU5F1* (*OCT4*), *TDGF1*, *DNMT3B*, *GABRB3* and *GDF3* were between 100 and 10,000 times upregulated compared with fibroblasts (Fig 1C) and immunocytochemical (ICC) analysis demonstrated the presence of the pluripotency markers OCT4, NANOG, TRA1-60, TRA1-81, SSEA3 and SSEA4 at the protein level (Fig 1D). Finally, in vitro differentiation followed by ICC analysis with the mesodermal marker smooth muscle actin (SMA), the endodermal marker alpha-feto protein (AFP) and the ectodermal marker beta-III-tubulin (TUJI) demonstrated the differentiation potential into all three germ layers (Fig 1E).

2. Materials and methods

Unless otherwise stated, consumables and reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.1. Establishment of iPSC

A skin biopsy was obtained from a 28-year old woman carrying a R406W mutation in MAPT. The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (RH), and written informed consent was obtained from the donor. 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). 1×10^5 fibroblasts were electroporated with a total of 1 μg of episomal plasmids containing *hOCT4* with or without a short hairpin

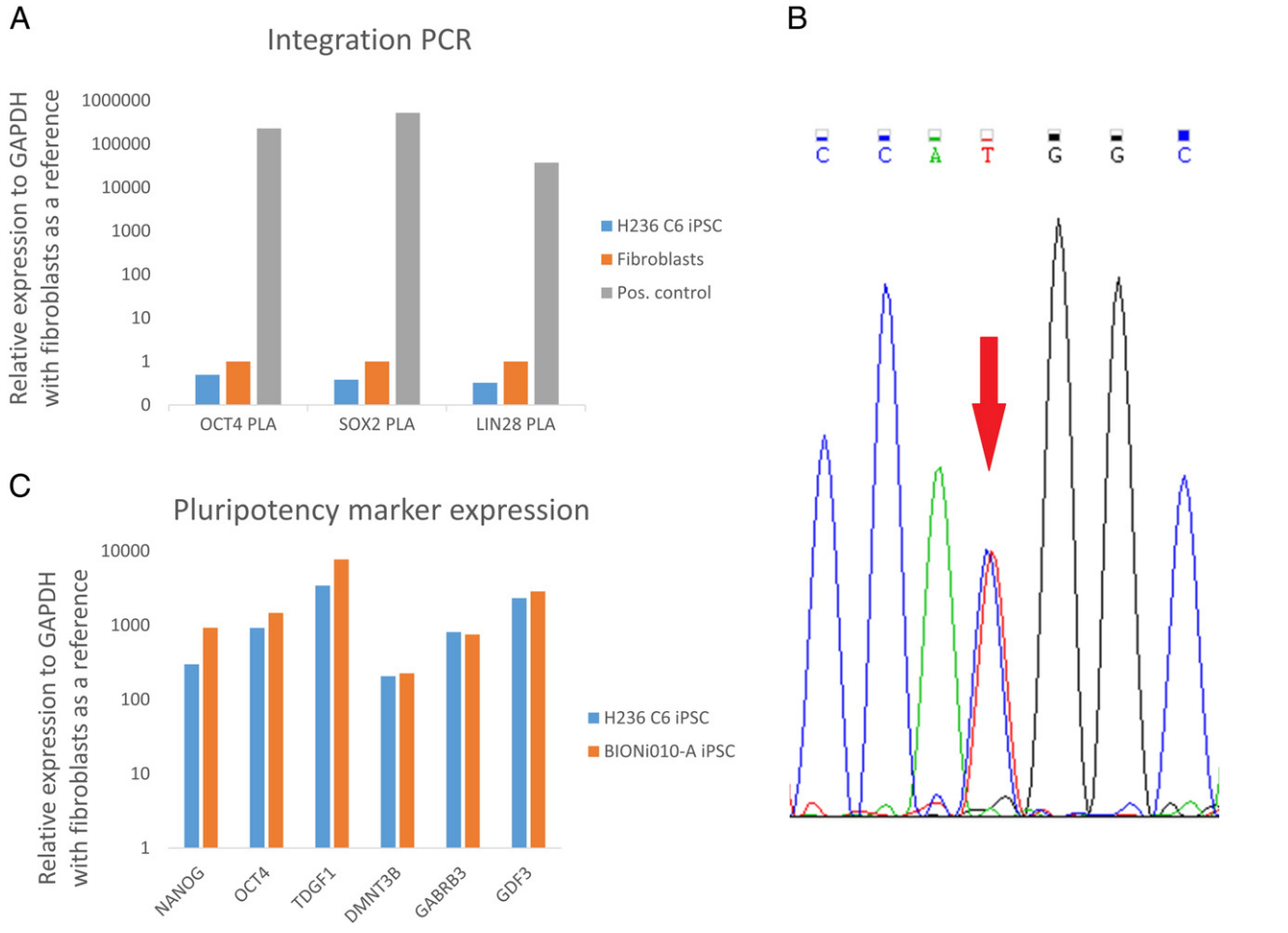

Fig. 1. A. Integration analysis. Quantitative PCR (qPCR) on genomic DNA from H236 C6 induced pluripotent stem cells (iPSC), fibroblasts and a pool of fibroblasts electroporated with episomal plasmids (positive control) with plasmid-specific primers of *hOCT4*, *hSOX2*, and *hLIN28*. Data is shown as the fold change ($2^{-\Delta\Delta Ct}$) with *GAPDH* and fibroblasts as references. B. Sequencing of mutation. Sequencing of exon 13 of the *MAPT* gene in H236 C6 induced pluripotent stem cells showing a c.1216C>T substitution in one of the alleles marked with a red arrow. C. Pluripotency expression analysis. Quantitative reverse-transcriptase PCR (qRT-PCR) expression analyses on cDNA from H236 C6 induced pluripotent stem cells (iPSC), fibroblasts and the iPSC line BIONi010-A (Rasmussen et al., 2014) as a positive control with the endogenous pluripotency genes *NANOG*, *POU5F1* (*OCT4*), *TDGF1*, *DNMT3B*, *GABRB3* and *GDF3*. Relative expression is shown as the fold change (2-ΔΔCt) with *GAPDH* and fibroblasts as references. D. Immunofluorescence staining. Immunocytochemical detection of H236 C6 induced pluripotent stem cells with the pluripotency markers OCT3/4, TRA1-81, NANOG, TRA1-60, SSEA3, and SSEA4. Scale bars correspond to 400 μm. E. *In vitro* differentiation. Immunocytochemical staining of plated embryoid bodies (EBs) from H236 C6 induced pluripotent stem cells on day 28 with smooth muscle actin (SMA), alpha-fetoprotein (AFP) and betaIII-tubulin (TUJI). Scale bars correspond to 100 μm. F. Karyotyping. Representative karyotype of H236 C6 induced pluripotent stem cells.

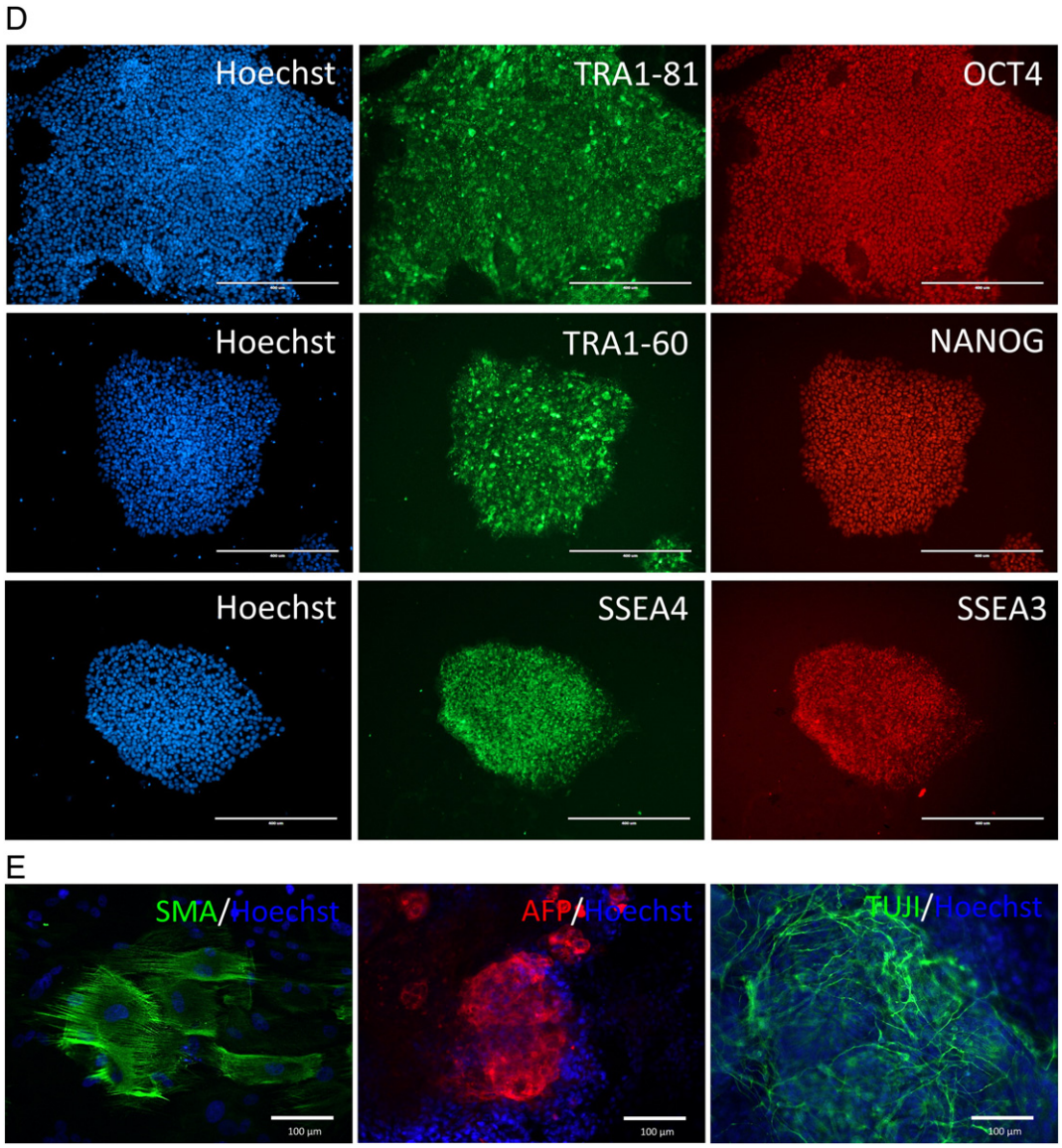

Fig. 1 (*continued*).

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, Carlsbad, CA, USA). One week after electroporation, the fibroblasts were trypsinized and split 1:2 onto hESC-qualified Matrigel-coated dishes (BD Biosciences, Franklin Lakes, NJ, USA) and cultured in mTeSR1 medium (Stem Cell Technologies, Vancouver, BC, Canada) in 5% O_2, 5% CO_2 in N_2. After four weeks, primary iPSC colonies were dissected out manually, transferred to new Matrigel-coated dishes, and cultured in mTeSR1. The iPSC lines were split 1:6 every 5–6 days with Dispase (Stem Cell Technologies, Vancouver, BC, Canada). At passage 10, the iPSC lines were harvested for subsequent analyses or frozen in liquid nitrogen in mTeSR1 containing 10% DMSO.

2.2. Integration analysis

DNA was purified from iPSCs, fibroblasts and a pool of fibroblasts electroporated with episomal plasmids as a positive control using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). qPCR analysis was carried out with the primers *OCT4-pla*, *SOX2-pla* and *LIN-28-pla*, which are specific to the three plasmids (Okita et al., 2011) and data was analyzed using the $2^{-\Delta\Delta Ct}$ method relative to *GAPDH* with fibroblasts as a reference (set to 1).

2.3. Pluripotency expression analysis

Total RNA was purified from iPSCs, fibroblasts and the iPSC line BIONi010-A (Rasmussen et al., 2014) as a positive control using RNeasy mini kit (Qiagen, Hilden, Germany). Conversion to cDNA was performed with RevertAid First Strand cDNA synthesis kit (Thermo Scientific, Waltham, MA, USA). qPCR analysis was carried out using the TaqMan primers *GAPDH Hs03929097_g1*, *NANOG Hs02387400_g1*, *POU5F1 Hs00999632_g1*, *TDGF1 Hs02339497_g1*, *DMNT3B Hs00171876_m1*, *GABRB3 Hs00241459_m1 and GDF3 Hs00220998_m1* (Thermo Scientific, Waltham, MA, USA; International Stem Cell Banking Initiative). Data was analyzed using the $2^{-\Delta\Delta Ct}$ method relative to *GAPDH* with fibroblasts as a reference (set to 1).

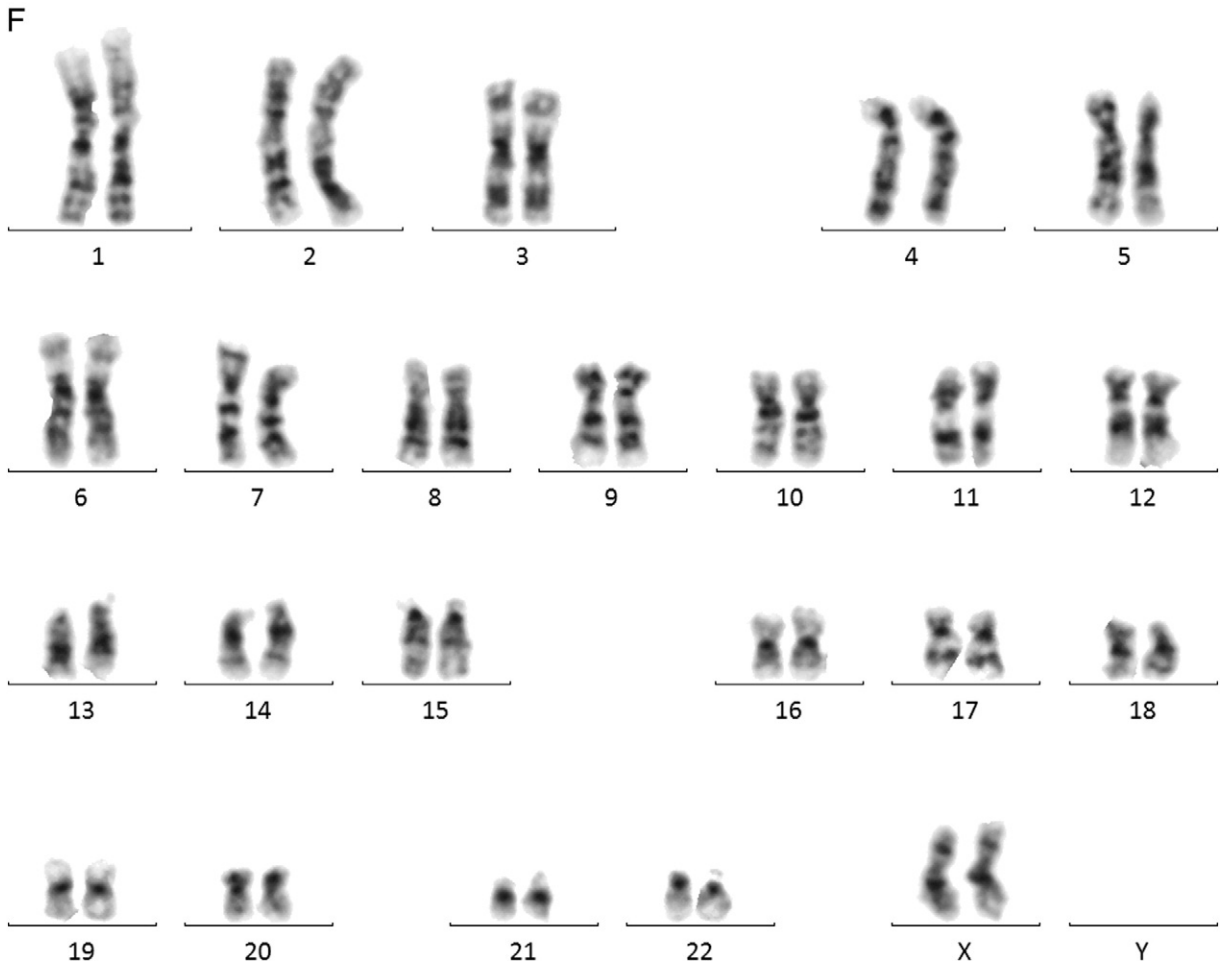

Fig. 1 (*continued*).

2.4. Sequencing of mutation

Sanger sequencing of exon 13 of the *MAPT* gene was carried out in an ABI PRISM 310 Genetic Analyzer using the primers MAPT_Ex13Fw ctggtctttctctggcactt and MAPT_Ex13Rv accaattaaccgaactgcg (NM_001123066.3; Lindquist et al., 2008).

2.5. Immunofluorescence staining

iPSCs were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min and stained by standard immunofluorescence procedures. The primary antibodies (Table 1) were visualized with the secondary antibodies Alexa 488 or Alexa 594 diluted 1:400 (Life technologies, Carlsbad, CA, USA) and counterstained with Hoechst bisbenzimide 33258. Images were acquired on a Leica DMRB-fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

2.6. In vitro differentiation

Embryoid body (EB) formation was performed by transferring Dispase-treated clumps of iPSC to ultra-low attachment plates (Corning, Corning, NY, USA) in mTeSR1. After 2 days of culture, the medium was changed to DMEM/F12 containing 20% knockout serum replacement (Life Technologies, Carlsbad, CA, USA), 1× non-essential amino acid, 2 mM L-glutamine, 0.1 mM 2-mercaptoethanol and 1% pen/strep. After 7 days, the EBs were plated on 0.1% gelatin-coated culture dishes and cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 1% pen/strep for up to three weeks. The cells were fixed for 15 min

Table 1
Antibodies used for immunocytochemistry.

	Antibody and host species	Dilution	Company and catalog number
Pluripotency	Rabbit anti-NANOG	1:500	Peprotech, 500-P236
	Goat anti-OCT4	1:500	Santa Cruz, sc-8628
	Rat anti-SSEA3	1:100	Biolegend, 330302
	Mouse anti-SSEA4	1:100	Biolegend, 330402
	Mouse anti-Tra-1-81	1:200	Biolegend, 330702
	Mouse anti-Tra-1-60	1:200	Biolegend, 330602
In vitro differentiation	Mouse anti-smooth muscle actin (SMA)	1:500	DAKO, M0851
	Rabbit anti-alpha-1-fetoprotein (AFP)	1:500	DAKO, A0008
	Mouse anti-Beta-III-tubulin (TUJI)	1:4000	Sigma-Aldrich, T8660

in 4% PFA for ICC analyses with the antibodies TUJI, SMA and AFP (Table 1).

2.7. Karyotyping

iPSCs and fibroblasts were treated for 45 min with KaryoMAX Colcemid (Life Technologies, Carlsbad, CA, USA) and harvested in fresh fixative containing 25% acetic acid and 75% methanol. Karyotyping was performed on G-banded metaphase chromosomes using standard cytogenetic procedures.

3. Verification and authentication

Karyotyping was performed at the Center for Applied Human Molecular Genetics, Kennedy Center, Glostrup, Denmark. At least 10 metaphases were analyzed per sample with an approximate resolution of 550 to 600 bands per haploid genome. The results showed a normal 46, XX karyotype, free of any discernible abnormalities (Fig. 1F). iPSC line identity and purity was confirmed by sequencing of the *MAPT* gene (Fig. B) and ICC with pluripotency markers (Fig. D).

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References

International Stem Cell Banking Initiative, 2009. Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. Stem Cell Rev. 5, 301–314.

Lindquist, S.G., Holm, I.E., Schwartz, M., Law, I., Stokholm, J., Batbayli, M., Waldemar, G., Nielsen, J.E., 2008. Alzheimer disease-like clinical phenotype in a family with FTDP-17 caused by a MAPT R406W mutation. Eur. J. Neurol. 15, 377–385.

Okita, K., Matsumura, Y., Sato, Y., Okada, A., Morizane, A., Okamoto, S., Hong, H., Nakagawa, M., Tanabe, K., Tezuka, K., Shibata, T., Kunisada, T., Takahashi, M., Takahashi, J., Saji, H., Yamanaka, S., 2011. A more efficient method to generate integration-free human iPS cells. Nat. Methods 8, 409–412.

Rasmussen, M.A., Holst, B., Tümer, Z., Johnsen, M.G., Zhou, S., Stummann, T.C., Hyttel, P., Clausen, C., 2014. Transient p53 suppression increases reprogramming of human fibroblasts without affecting apoptosis and DNA damage. Stem Cell Rep. 9, 404–413.