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Lab Resource: Single Cell Line

Reprogramming of human olfactory neurosphere-derived cells from olfactory mucosal biopsies of a control cohort

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A B S T R A C T

Human olfactory neurosphere-derived (ONS) cells are derived from the olfactory mucosa and display some progenitor- and neuronal cell-like properties, making them useful models of neurological disorders. However, they lack several important characteristics of true neurons, which can be overcome using induced pluripotent stem cell (iPSC) -derived neurons. Here we describe, for the first time, the generation and validation of an iPSC line from an olfactory biopsy from a control cohort member. This data lays the groundwork for future reprogramming of ONS cells, which can be used to generate neuronal models and compliment current ONS cell-based investigations into numerous neurological disorders.

Resource Table:

Unique stem cell line identifier	*AIBNi013-A*
Alternative name(s) of stem cell line	*ONS-derived Control iPSC, Control HL iPSC*
Institution	*Australian Institute for Bioengineering and Nanotechnology*
Contact information of distributor	*Professor Ernst J Wolvetang: e.wolvetang@uq.edu.au*
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info required for human ESC or iPSC	*Age: 25* *Sex: Male* *Ethnicity if known: Unknown*
Cell Source	*Human olfactory neurosphere derived cells (ONS cells)* *GRIDD Neuro Bank ID # 100,150,004*
Clonality	*Clonal*
Method of reprogramming	*Non-integrative Sendai virus delivery of OCT4, SOX2, KLF4 and c-MYC transgenes*
Genetic Modification	*No*
Type of Genetic Modification	*N/A*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*PCR*
Associated disease	*None*
Gene/locus	*N/A*
Date archived/stock date	*09/04/2019*
Cell line repository/bank	*https://hpscreg.eu/cell-line/AIBNi013-A*
Ethical approval	*UQ HREC 2,019,000,159 - Generation, differentiation and genetic manipulation of human induced pluripotent stem cells.* *GU HREC ESK/01/11/HREC – Olfactory biopsies in neurological disorders and diseases.*

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1. Resource utility

This represents the first time that induced pluripotent stem cells (iPSCs) have been generated from olfactory neurosphere-derived (ONS) cells that originate from olfactory mucosal biopsies, and lays the experimental groundwork for future ONS reprogramming endeavors. Establishing iPSCs from ONS control and disease cohorts will facilitate ongoing research into neurological conditions.

2. Resource details

Olfactory dysfunction manifests as a clinical or preclinical symptom in several neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease. Olfaction is orchestrated by olfactory mucosal cells located in the upper nasal cavity, where local stem cells continually regenerate olfactory sensory neurons throughout life (Doty, 2017). The olfactory mucosa contains olfactory axons from the peripheral nervous system as well as ensheathing glial cells and represents a direct link to the central nervous system. Olfactory mucosal samples may be obtained for *in vitro* investigations via a relatively non-invasive biopsy under local

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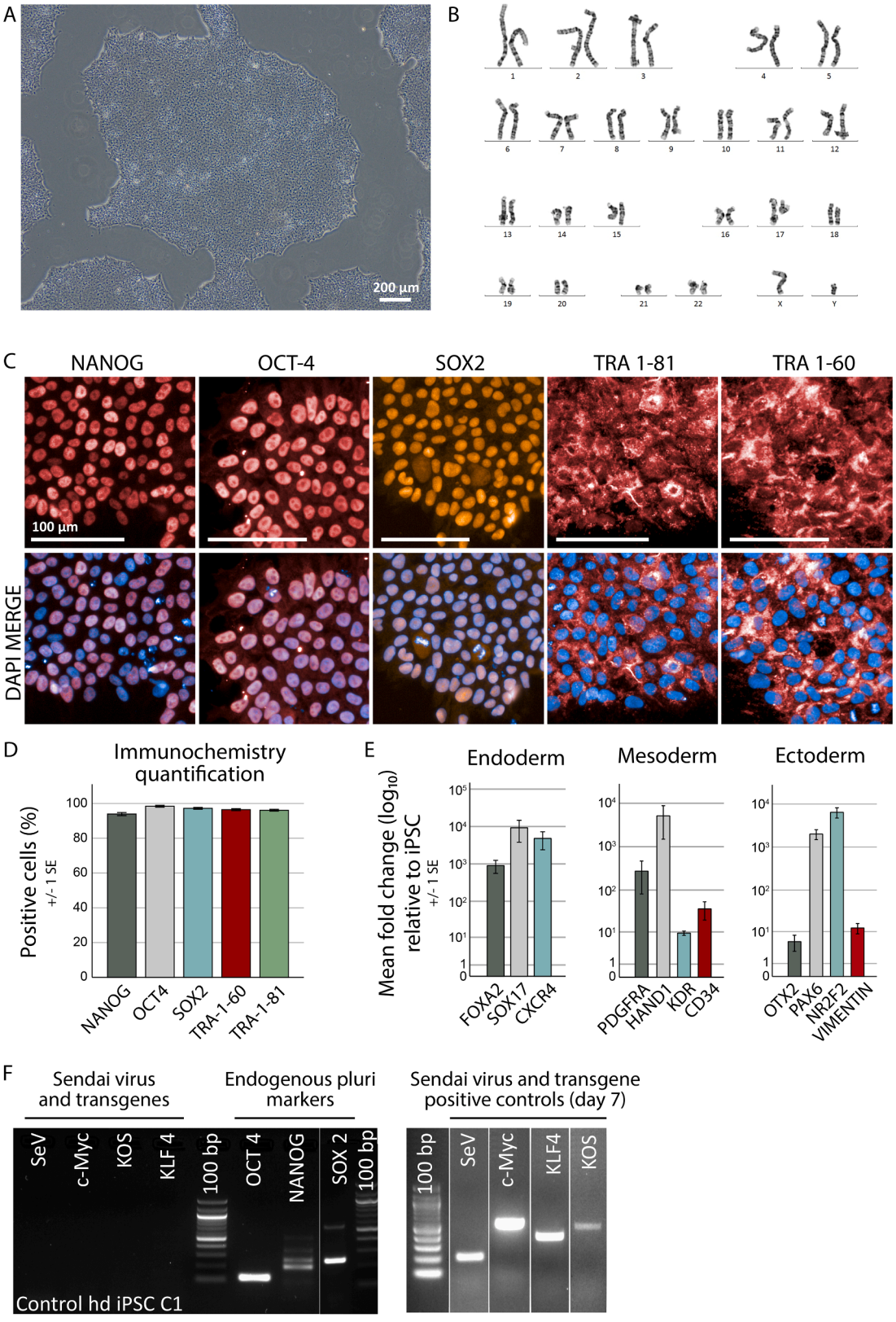

Fig. 1. Characterisation of control ONS-derived iPSCs. (A) Bright field image of representative iPSC morphology. Scale bar 200 µm. (B) Karyotype demonstrating normal 46XY banding pattern at 400bphs. (C) Immunochemistry demonstrated iPSCs were positive for NANOG, OCT-4, SOX2, TRA 1-81, and TRA 1-60, with nuclei counterstained with DAPI and scale bar 100 µm. (D) Quantification of immunochemistry shown in panel C. (E) qPCR analysis following directed differentiations demonstrated the potential for all three germ layers. Error bars +/- 1 SE. (F) PCR demonstrating loss of Sendai virus and transgenes, along with presence of endogenous pluripotency markers. Positive controls were harvested at day 7.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Compact flat colonies with a well-defined smooth edge, containing cells with a high nucleus to cytoplasm ratio and prominent nucleoli	Fig. 1, panel A
Phenotype	Qualitative analysis: Immunocytochemistry staining	Positive staining of pluripotency markers: OCT4, SOX2, NANOG, TRA-1–60, TRA-1–81	Fig. 1, panel C
	Quantitative analysis	Robust endogenous expression of OCT4, NANOG, SOX2, TRA 1–60 and TRA 1–81 in > 95% of cells	Fig. 1, panel D
Genotype	Karyotype (G-banding) and resolution	46XY, resolution 400bphs	Fig. 1, panel B
Identity	STR analysis	10 loci tested and matched to original cell line	Provided
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by Myco Alert Assay returned negative results with testing monthly	Not shown, available with author
Differentiation potential	Directed differentiation	Expression of endoderm markers (Sox17, Foxa2, Cxcr4), mesoderm markers (Pdgfra, Hand1, Kdr, CD34) and ectoderm markers (Otx2, Pax6, NR2F2, Vimentin).	Fig. 1, panel E
List of recommended germ layer markers	Germ layer expression validated by qPCR	Expression of endoderm markers (Sox17, Foxa2, Cxcr4), mesoderm markers (Pdgfra, Hand1, Kdr, CD34) and ectoderm markers (Otx2, Pax6, NR2F2, Vimentin).	Fig. 1, panel E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

anaesthetic, and provide a patient-specific model for neurological disorders (Féron et al., 1998). Once in culture, these olfactory mucosal cells may be proliferated as 'neurospheres' to promote the expansion of cells (olfactory neurosphere-derived, or ONS cells) that display some of the properties of progenitor- and neuronal-like cells. To date, ONS cells have been used as patient-specific models of ataxia telangiectasia, hereditary spastic paraplegia, Parkinson's disease, schizophrenia, epilepsy and autism (Stewart et al., 2013; Wali et al., 2018; Matigian et al., 2010).

Unlike iPSCs, ONS are easy and cost effective to maintain, though they do not display several key properties of true neurons, such as synapses and action potentials. To compliment the utility of ONS as a model for neurological diseases, ONS cells were reprogrammed to generate vector-free iPSCs. Reprogramming from ONS cells had not been previously attempted, thus we began with a control ONS line (ID 100150004) to eliminate confounding factors presented by disease phenotypes. CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher) was used to transduce the cell line, and while we experienced a lower reprogramming efficiency than the anticipated 0.01–1% reported in the manufacturer guidelines, we successfully generated an iPSC line displaying all the typical characteristics. While the lowest possible passage was used for reprogramming, the ONS line was passage 10 and we expect that this may have had a detrimental impact on reprogramming efficiency.

The generated iPSC line demonstrated typical morphology (Fig. 1A) and a normal karyotype (46XY, Fig. 1B) was observed at 400bphs. Immunochemistry for stem cell markers NANOG, OCT4, SOX2, TRA-1–81 and TRA-1–60 was used to validate pluripotency (Fig. 1C, D). Directed differentiations to each lineage confirmed germ layer potential, and when assessed by qPCR (Fig. 1E) showed upregulation of markers for endoderm (FOXA2, SOX17, CXCR4), mesoderm (PDGFRA, HAND1, KDR, CD34), and ectoderm (OTX2, PAX6, NR2F2, VIMENTIN) along with a downregulation of pluripotency markers NANOG, OCT4 and SOX2 (data not shown). To confirm generation of footprint free iPSCs, control ONS-derived iPSCs were tested for loss of the Sendai virus and associated transgenes, compared to positive controls harvested on day 7 of reprogramming. Endogenous pluripotency markers OCT4, NANOG and SOX2 were positive (Fig. 1F). Short tandem repeat (STR) analysis was used to confirm that the iPSC profile matched that of the control ONS donor (Supplementary data). Additional information about the characterisation and validation of this control ONS-derived iPSC line is provided in Tables 1 and 2. Collectively, this data demonstrates the successful generation and quality of a pluripotent hiPSC line from a member of the control ONS cohort.

3. Materials and methods

3.1. Reprogramming ONS to iPSCs

Human olfactory neurosphere derived (ONS) cells were previously obtained as nasal biopsies from the olfactory mucosa of healthy donors, with informed consent and ethical approval by Griffith University Human Ethics Committee. ONS cells were cultured in DMEM/F12 supplemented with 10% foetal bovine serum, 1X Glutamax and 1X non-essential amino acids. Reprogramming was carried out via transduction with non-integrating Sendai virus (SeV) and reprogramming transgenes OCT4, SOX2, KLF4 and c-MYC. Reprogramming was completed according to the manufacturer's instructions specific for fibroblasts, with modification of fibroblast medium to ONS medium. Cells were maintained in ReproTeSR until colony formation. Emerging colonies were individually selected, and medium was transitioned to mTeSR Plus (Stemcell Technologies). Subsequent passages were performed using 0.5 mM EDTA at a split ratio of 1:5 – 1:8 approximately every 5 days. Clumps were plated without ROCK inhibitor on hESC qualified Matrigel (Corning), and were maintained at 37 °C, 5% CO_2. All analysis was conducted passage 5 onwards.

3.2. Immunofluorescence staining

iPSCs were fixed with 4% PFA for 10 min at 4 °C before blocking and permeablisation for 1 h with 3% BSA and 0.1% TritonX-100 in PBS. Pluripotency markers as outlined in Table 2 were incubated overnight at 4 °C. Following PBS washes, secondary antibodies (Table 2) were

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Mouse Anti OCT4 IgG	1:100	Millipore Cat# MAB4419	RRID:AB_1977399
	Rabbit Anti SOX2 IgG	1:400	Cell Signaling Technology Cat# 23064	RRID:AB_2714146
	Mouse Anti NANOG IgG	1:2000	Cell Signaling Technology Cat# 4893	RRID:AB_10548762
	Mouse Anti Tra-1–60 IgG	1:200	Millipore Cat# MAB4360	RRID:AB_2119183
	Mouse Anti Tra-1–81 IgG	1:100	Millipore Cat# MAB4381	RRID:AB_177638
Secondary Antibodies	Goat Anti Mouse IgG H + L Alexa Fluor 647	1:500	Thermo Fisher Scientific Cat# A-21235	RRID:AB_2535804
	Goat Anti Mouse IgG H + L Alexa Fluor 488	1:500	Thermo Fisher Scientific Cat# A-11029	RRID:AB_2534088
	Goat Anti Rabbit IgG H + L Alexa Fluor 647	1:500	Thermo Fisher Scientific Cat# A-21245	RRID:AB_2535813
	Donkey Anti Mouse IgG H + L Alexa Fluor 568	1:500	Thermo Fisher Scientific Cat# A-10037	RRID:AB_2534013
	Primers			
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai transgenes (PCR)	Sendai virus (SeV) genome	181	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
	KOS	528	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG	
	KLF-4	410	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
	c-MYC	532	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
Endogenous pluripotency markers (PCR)	OCT-4	113	AGTTTGTGCCAGGGTTTTTG/ ACTTCACCTTCCCTCCAAC	
	Nanog	194	TTTGGAGCTGCTGGGGAAG/ GATGGGAGGAGGGGAGAGGA	
	SOX2	234	CATGTCCCAGCACTACCAGA/ GTCATTTGCTGTGGGTGATG	
Differentiation primers (qPCR)	SOX17 (endoderm)	94	GTGGACCGCACGGAATTTG/ GGAGATTCACACCGGAGTCA	
	FOXA2 (endoderm)	83	GGAGCAGCTACTATGCAGAGC/ CGTGTTCATGCCGTTCATCC	
	CXCR4 (endoderm)	61	GCCTTACTACATTGGGATCAG/ CCCTTGCTTGATGATTTCCA	
	PDGFRA (mesoderm)	92	GTCTTCTCACAGGGCTGAG/ TGAATTCAGCTGCACAACC	
	HAND1 (mesoderm)	170	CCATGCTCCACGAACCCTTC/ CCTGGCGTCAGGACCATAG	
	KDR (mesoderm)	81	GTCACTTTGTGCAAGATACCC/ GTAAAGCCCTTCTTGCTGTC	
	CD34 (mesoderm)	89	CCAACAGAACAGAAATTTCCAG/ CTCAGTGAAATCTAGGATCCC	
	Otx2 (ectoderm)	81	CCAGACATCTTCATGCGAG/ TCGATTCTTAAACCATACCTGC	
	Pax6 (ectoderm)	320	ACACACTTGAGCCATCACCA/ TTCCACGGGGCTCGAATATG	
	NR2F2 (ectoderm)	151	TCATGGGTATCGAGAACATTTGC/ TTCAACACAAACAGCTCGCTC	
	Vimentin (ectoderm)	81	TCCACGAAGAGGAAATCCA/ CAGGCTTGGAAACATCCAC	
House-Keeping Gene (qPCR)	GAPDH	66	AGCCACATCGCTCAGACAC/ GCCCAATACGACCAAATCC	

incubated for 1hr at room temperature. Nuclei were counterstained with Hoechst.

3.3. Germ lineage differentiations

Directed differentiation was used to confirm potential for generation of three germ layers. For ectoderm differentiation, neural induction medium (1:1 DMEM/F12 and Neurobasal medium, 0.5X N2, 0.5X B-27, 1X GlutaMax, 0.5X NEAAs, 2.5 μg/mL insulin and 50 μM β-mercaptoethanol) with dual SMAD inhibition (10 μM SB431542 and 0.1 μM LDN-193189) was fed for 10 days. Endoderm differentiation used STEMdiff Definitive Endoderm Kit (Stemcell Technologies) for 5 days and mesoderm was fed with RPMI supplemented with 1X B-27 and 5 μM CHIR for 5 days. Differentiations were harvested for RNA and analysed by qPCR.

3.4. Quantitative PCR (qPCR)

RNA was extracted using Nucleospin RNA extraction kit (Macherey-Nagel) and cDNA was synthesised using BioRad iScript cDNA Synthesis kit. All qPCR reactions were performed in triplicate using PowerUp SYBR Green Master Mix (Thermo Fisher) and a BioRad CXF96 Real Time machine. Cycle conditions were 95 °C for 2 min, followed by denaturation (95 °C for 15 s), annealing (55–60 °C for 15 s) and extension (72 °C for 1 min) for 40 cycles. Results were expressed as double delta CT. Primers are listed in Table 2.

3.5. Endpoint PCR

PCR was conducted on cells to confirm loss of reprogramming transgenes. Positive controls were harvested 21 days after transduction. RNA extraction and cDNA synthesis were as described above, and GoTaq Green Master Mix was used to carry out the PCRs. Primers against transgenes KOS, KLF-4, c-MYC, and SeV are listed in Table 2.

3.6. Karyotyping

The iPSC line was karyotyped by Sullivan Nicolaides Pathology (Bowen Hills, Queensland), at a resolution of 400 bands per haploid set (bphs).

3.7. Short tandem repeat (STR) analysis

DNA was extracted from the iPSCs and the donor's corresponding ONS line for STR analysis (GenePrint-10) by the Australian Genome Research Facility in Melbourne.

3.8. Mycoplasma testing

Culture medium routinely underwent mycoplasma testing using MycoAlert Assay.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102527.

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