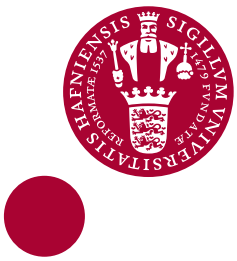

Generation and characterization of three isogenic induced pluripotent stem cell lines from a patient with Bardet-Biedl syndrome and homozygous for the BBS5 variant

Hey, Caroline Amalie Brunbjerg; Larsen, Lasse Jonsgaard; Tümer, Zeynep; Brøndum-Nielsen, Karen; Grønskov, Karen; Hjortshøj, Tina Duelund; Møller, Lisbeth Birk

Published in:
Stem Cell Research

DOI:
10.1016/j.scr.2019.101594

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Stem Cell Research 41 (2019) 101594

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab resource: Stem Cell Line

Generation and characterization of three isogenic induced pluripotent stem cell lines from a patient with Bardet-Biedl syndrome and homozygous for the *BBS5* variant

Caroline Amalie Brunbjerg Hey[a], Lasse Jonsgaard Larsen[a], Zeynep Tümer[a,b], Karen Brøndum-Nielsen[a], Karen Grønskov[a], Tina Duelund Hjortshøj[a], Lisbeth Birk Møller[a,*]

[a] Kennedy Center, Department of Clinical Genetics, Copenhagen University Hospital, Rigshospitalet, Gl. Landevej 7, 2600 Glostrup, Denmark
[b] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200N Copenhagen, Denmark

A B S T R A C T

Bardet-Biedl syndrome (BBS), an autosomal recessive disease, is associated with non-functional primary cilia. BBS5 is part of the protein complex termed the BBSome. The BBSome associates with intra flagellar transport (IFT) particles and mediates trafficking of membrane proteins in the cilium, a process important for cilia-mediated signal transduction. Here we describe the generation of three induced pluripotent stem cell (iPSC) lines, KCi003-A, KCi003-B and KCi003-C from a patient with BBS and homozygous for the disease causing variant c.214G > A, p.(Gly72Ser) in BBS5. The iPSC lines can be used for investigation of IFT in different cell types differentiated from the iPSC line.

Resource table

Unique stem cell lines identifier	KCi003-A KCi003-B KCi003-C
Alternative names of stem cell lines	BBS5 cl. 3A (KCi003-A) BBS5 cl. 4A (KCi003-B) BBS5 cl. 5A (KCi003-C)
Institution	Kennedy Center, Rigshospitalet
Contact information of distributor	Lisbeth Birk Møller, Lisbeth.Birk.Moeller@regionh.dk
Type of cell lines	iPSC
Origin	Human
Cell Source	Dermal fibroblasts (KC-85)
Clonality	Clonal
Method of reprogramming	Nucleofection with non-integrating episomal plasmids carrying *OCT3/4, SOX2, KLF4, L-MYC, LIN28* and *shP53*
Multiline rationale	Isogenic clones
Genetic Modification	NA
Type of Modification	NA
Associated disease	Autosomal recessive Bardet-Biedl syndrome
Gene/locus	*BBS5*, Chr2: g.170344321G > A, p.(Gly72Ser), homozygous Ref sequence: NM_152384.2
Method of modification	NA
Name of transgene or resistance	NA
Inducible/constitutive system	NA
Date archived/stock date	01–03–2019
Cell line repository/bank	NA
Ethical approval	The study was approved by the regional scientific ethical committee in the Capital Region of Denmark (H-3-2014-140). Written informed consent was obtained from the patients.

1. Resource utility

To gain further insight into the complicated mechanisms of the ciliopathy disorder, Bardet-Biedl syndrome (BBS), and the implications of variants discovered in the proteins of the BBSome complex, induced pluripotent stem cells (iPSC) were generated from a patient homozygous for the *BBS5* variant: c.214G > A, p.(Gly72Ser).

2. Resource details

BBS is a autosomal recessive disorder with an estimated incidence between 1:13,500 and 1:160,000 depending on the geographic location (Forsythe and Beales, 2013; Hjortshøj et al., 2010). BBS5 is part of the protein complex termed the BBSome. The BBSome associates with intra flagellar transport (IFT) particles and mediates trafficking of membrane proteins in the cilium, a process important for primary cilia-mediated movement and signal transduction (Mourão et al., 2016; Nachury et al.,

* Corresponding author.
E-mail address: Lisbeth.Birk.Moeller@regionh.dk (L.B. Møller).

https://doi.org/10.1016/j.scr.2019.101594
Received 22 July 2019; Received in revised form 15 September 2019; Accepted 19 September 2019
Available online 04 November 2019

2007). The primary cilium, a membrane protrusion present on most quiescent cells. The cilium is important for coordinating certain cellular processes. Due to non-functional cilia in BBS patients, the patients are characterized by systemic manifestations including obesity, renal cysts, polydactyly, retinal dystrophy, learning difficulties and hypogonadism.

A fibroblasts culture were established from a skin biopsy obtained from a patient with Bardet-Biedl syndrome homozygous for the *BBS5* variant: c.214G > A, p.(Gly72Ser). Low-passage fibroblasts were reprogramed into iPSC using episomal plasmids coding for the human genes *OCT3/4, SOX2, KLF4,* L*-MYC, LIN28* and a short hairpin RNA targeting p53, resulting in generation of three iPSC lines KCi003-A, KCi003-B and KCi003-C (Table 1). Sanger sequencing showed presence of the variant *BBS5*: c.214G > A, p.(Gly72Ser) in all three generated iPSC lines (Fig. 1A and Supl. Fig. 1A and B) and STR analysis comparing 22 sites between the original fibroblast culture, KCi003-A, KCi003-B and KCi003-C gave 100% identity match confirming the origin of KCi003-A, KCi003-B and KCi003-C (Table 2, supplementary file). The karyotype was confirmed to be normal (46,XY) in all three iPSC lines (Fig. 1B). Expression of pluripotency-related genes *OCT* 3/4*, NANOG, SOX2* and *TRA*-1–60 was investigated using immunofluorescence microscopy. Nuclear localization of OCT3/4, NANOG and SOX2 was confirmed and TRA-1–60 was visible in the membrane of the investigated colonies of KCi003-A, KCi003-B and KCi003-C (Fig. 1C). Expression level of seven pluripotency-associated genes was investigated by real-time quantitative RT-PCR (qRT-PCR) using taqman probes and primers (Table 3). Similar levels of expression were obtained for KCi003-A, KCi003-B, KCi003-C and a control iPSC line (Fig. 1D), whereas fibroblasts had zero expression of the tested genes (not shown). The absence of genomic integrated plasmids was validated by quantitative PCR (q-PCR) using genomic DNA from KCi003-A, KCi003-B and KCi003-C together with SYBR green and primers specific for the reprogramming plasmids (Pla). DNA from fibroblasts 72 h post transfection was used as a positive control. Quantitative PCR using primers specific for the coding sequence (CDS) of *OCT, SOX2, KLF4* and *LIN28* (Table 3) confirmed the presence of the endogenous genes in the three iPSC clones and the control IPSC. As these primers detect both endogenous and plasmid-derived genes (Okita et al., 2011) the highest signal was obtained in DNA from fibroblasts, 72 h post transfection (Fig. 1E). The ability to form cells of all three germ layers was tested by embryoid body formation followed by adherent culture and immunofluorescence analysis of smooth muscle actin (SMA), α-fetoprotein (AFP) and βIII-tubulin (βtub). KCi003-A, KCi003-B and KCi003-C show clear expression of SMA, AFP and βtub, confirming the ability to differentiate into cells of all three germ layers (Fig. 1F). Altogether these results confirm the origin and pluripotent state of KCi003-A, KCi003-B and KCi003-C.

3. Materials and methods

3.1. Cell culture and reprogramming

Fibroblast cells were maintained at 37 °C and 5%-CO_2 in DMEM-F12 + GlutaMAX with 10% foetal bovine serum and 1% penicillin-streptomycin (all Gibco). Addgene episomal plasmids #27077, #27078, #27080 (1.25 ng of each for 5×10^5 cells) were transfected into low passage cells using Primary Mammalian Fibroblasts buffer (Lonza) and program V-024 on Amaxa Nucleofector™ 2b. Cells were seeded on gelatine coated dishes post transfection, in culture medium without antibiotics for the first 24 h. Cells were transferred to ESC grade Matrigel (Corning) 6-well coated dishes on day 6 post transfection (50–80 $\times 10^3$ cells per 6-well) and cultured in mTeSR1 (Stemcell Technologies) with a gas composition of 5% CO_2, 5% O_2, 90% N_2 at 37 °C. Gentle cell Dissociation Reagent (Stemcell Technologies) was used for passaging and cells were frozen in mTeSR1 supplemented with 10% DMSO (Sigma-Aldrich).

3.2. DNA isolation and analyses

To purify DNA, the kit DNeasy Blood and Tissue kit (QIAGEN) was used. DNA was used for plasmid integration analysis, genotyping and Short tandem Repeat (STR) analysis. Primers used for integration, genotyping and STR analysis are listed in Table 3. SYBR green reagents were used for integration analysis and the ΔΔCT method applied with amounts normalized to GAPDH. STR analysis was carried out using Elucigene QST*R PLUSv2.

3.3. Karyotyping

Cells (passage 10 or higher) were treated with KaryoMAX colcemid for 45 min, dissociated, treated with hypotonic solution and fixed in fresh 75% methanol and 25% acetic acid. Two metaphase chromosomes stained with Giemsa were investigated per clone.

3.4. Gene expression analysis

RNA was harvested using the RNeasy kit (QIAGEN). RNA was DNase treated with DNase I (Invitrogen) before cDNA synthesis with high capacity cDNA kit (Applied Biosystems). Taqman probes are listed in Table 3. Data was analysed using the ΔΔCT method. Amounts were normalized to GAPDH and a control iPSC.

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
KCi003-A	KCi003-A	Male	39	Somali	*BBS5,* Chr2: g.170344321G > A, p.(Gly72Ser)	Bardet-Biedl syndrome
KCi003-B	KCi003-B	Male	39	Somali	*BBS5,* Chr2: g.170344321G > A, p.(Gly72Ser)	Bardet-Biedl syndrome
KCi003-C	KCi003-C	Male	39	Somali	*BBS5,* Chr2: g.170344321G > A, p.(Gly72Ser)	Bardet-Biedl syndrome

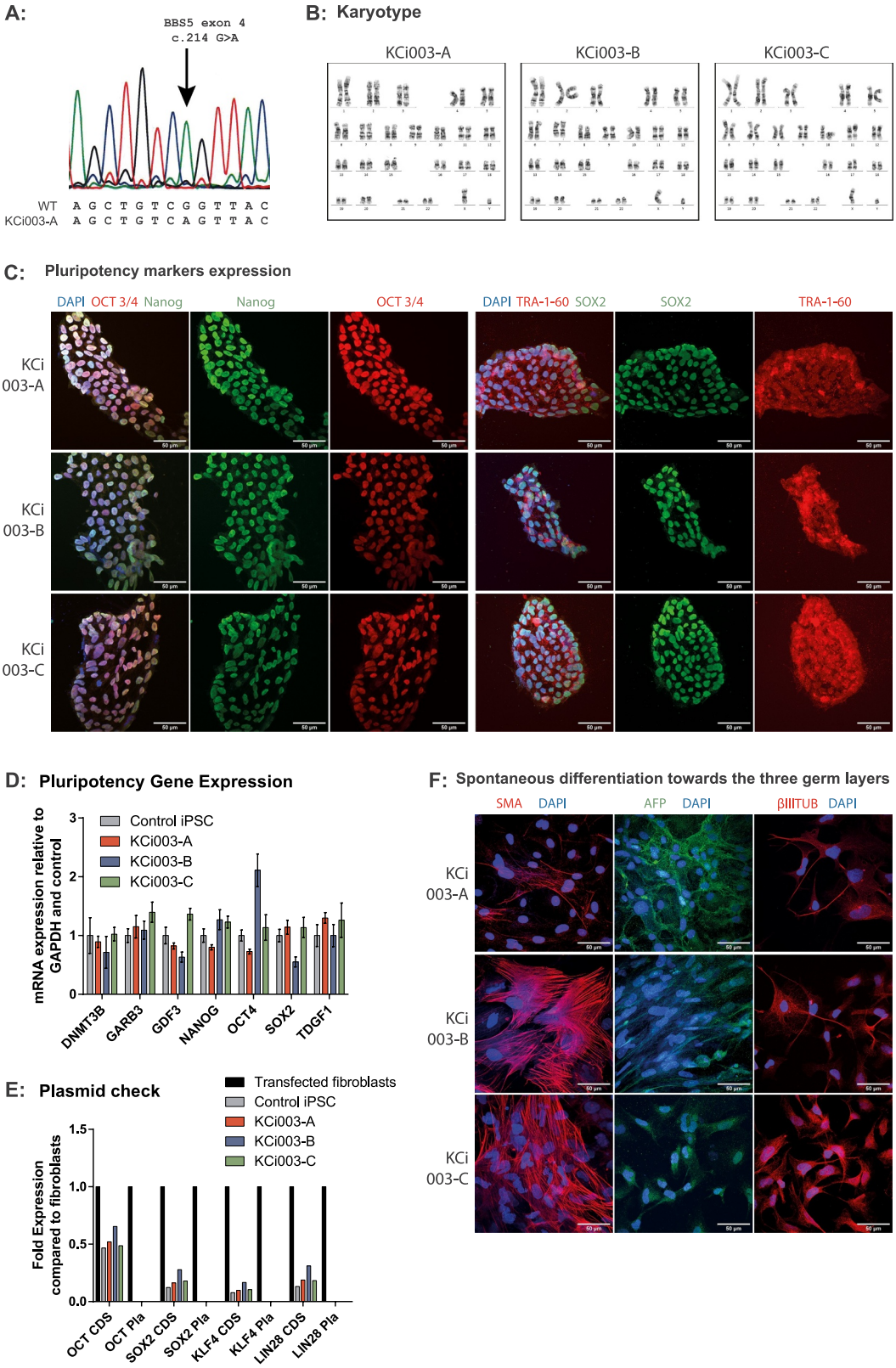

Fig. 1. Characterization and validation of three BBS5 iPSC lines.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal ES-like morphology	Not shown
Phenotype	Immunocytochemistry	Positive for cell surface markers; OCT4, NANOG, SOX2, TRA-1-60	Fig. 1 panel C
	Quantitative Real-Time PCT (TaqMan probes; Applied Biosystems 7500 Fast system)	Positive for; OCT4, NANOG, SOX2, TDGF1, DNMT3B, GARB3 and GDF3	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46,XY, Resolution 450–500	Fig. 1 panel B
Identity	DNA Profiling STR analysis	DNA Profiling not performed	NA
		22 sites were tested. 100% identity match between parental fibroblasts and KCi003-A, KCi00-B and KCi003-C.	Submitted in archive with journal
Variant analysis	Sanger sequencing	*BBS5*: c.214G > A, p.(Gly72Ser) homozygous	Fig. 1 panel A and Supplementary Fig. 1A and B
	Southern Blot OR WGS	NA	NA
Microbiology and virology	Mycoplasma	Mycoplasma testing by RT-PCR (negative)	Supplementary Files 1, 2 and 3
Differentiation potential	Embryoid body formation followed by spontaneous differentiation		
	Presence of the proteins α-smooth muscle actin (SMA), α-fetoprotein (AFP) and βIII-tubulin (βtub) were used to confirm formation of the three germ layers.	Fig. 1 panel F	
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	NA
Genotype additional info	Blood group genotyping	NA	NA
	HLA tissue typing	NA	NA

3.5. In vitro spontaneous differentiation

Spontaneous differentiation was initiated by embryoid body formation in suspension culture for one week followed by adherent culture for two weeks. The first day of suspension culture, cell aggregates were performed in mTeSR1 with ROCK inhibitor. The following 6 days the cells were cultured in differentiation medium (DMEM-F12 + GlutaMAX (Gibco), 20% knock-out serum replacement (Gibco), 1x non-essential amino acids (Sigma), 0,1 mM 2-mecaptoethanol (Sigma) and 1% penicillin-streptomycin (Gibco). For adherent culture, the cell aggregates were cultured in fibroblast medium on gelatine (Sigma) coated dishes. Morphology was observed, and at the end of the protocol cells were analysed using immunocytochemistry (see below).

3.6. Immunocytochemistry

To fixate the cells, incubation with 4% paraformaldehyde (Hounisen) at RT for 15 min was carried out. Then, the cells were incubated with 0,2% TritonX-100 in PBS for 15 min to permeabilize the cells. The cells were treated with blocking buffer consisting of 3% BSA (Tocris) and 0,2% TritonX-100 (Sigma) in PBS for one hour. Primary antibody incubation was carried out for two hours at RT or 5 °C overnight. Secondary antibody incubation was 45 min at RT. DAPI diluted in PBS was used to visualize nuclei. All used antibodies were diluted in blocking buffer and antibody details are found in Table 3.

Table 3
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency Marker	Rabbit anti-NANOG	1:500	PeproTech Cat# 500-P236, RRID: AB_1268805
Pluripotency Marker	Mouse anti-OCT3/4	1:200	Santa Cruz Biotechnology Cat# sc5279, RRID: AB_628051
Pluripotency Marker	Rabbit anti-SOX2	1:200	ThermoFisher Cat# PA1-094, RRID: AB_ 2539862
Pluripotency Marker	Mouse anti–TRA–1–60	1:200	BioLegend Cat# 330,602, RRID: AB_1186144
Differentiation Marker, Mesoderm	Mouse anti- α-smooth muscle actin (SMA)	1:500	Dako Cat# M0851, RRID: AB_2223500
Differentiation Marker, Endoderm	Rabbit anti-α-fetoprotein (AFP)	1:500	Dako Cat# A0008, RRID: AB_2650473
Differentiation Marker, Ectoderm	Mouse anti-βIII tubulin (βtub)	1:4000	Sigma-Aldrich Cat# T8660, RRID: AB_477590
Secondary antibody	Alexa Flour Goat Anti-Rabbit 488	1:800	Life Technologies Cat# A11008 RRID: AB_143165
Secondary antibody	Alexa Flour Donkey Anti-Mouse 546	1:800	Life Technologies Cat# A10036 RRID: AB_2534012
Secondary antibody	Alexa Flour Rabbit Anti-Rat 488	1:800	Molecular Probes Cat# A-21210 RRID: AB_2535796

Primers

	Target	Forward/Reverse primer (5′ – 3′)
Episomal Plasmids (qPCR)	OCT3/4 Plasmid	CATTCAAACTGAGGTAAGGG/ TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	OCT3/4 Endogenous (CDS)	CCCCAGGGCCCCATTTTGGTACC/ ACCTCAGTTTGAATGCATGGGAGAGC
Episomal Plasmids (qPCR)	KLF4 Plasmid	CCACCTCGCCTTACACATGAAGA/ TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	KLF4 Endogenous (CDS)	ACCCATCCTTCCTGCCCGATCAGA/ TTGGTAATGGAGCGGCGGGACTTG
Episomal Plasmids (qPCR)	SOX2 Plasmid	TTCACATGTCCCAGCACTACCAGA/ TTTGTTTGACAGGAGCGACAAT
Endogenous (qPCR)	SOX2 Endogenous (CDS)	TTCACATGTCCCAGCACTACCAGA/ TCACATGTGTGAGAGGGGCAGTGTGC
Episomal Plasmids (qPCR)	L-MYC Plasmid	GGCTGAGAAGAGGATGGCTAC/ TTTGTTTGACAGGAGCGACAAT
Endogenous (qPCR)	L-MYC Endogenous (CDS)	GCGAACCCAAGACCCAGGCCTGCTCC/ CAGGGGGTCTGCTCGCACCGTGATG
Episomal Plasmids (qPCR)	LIN28 Plasmid	AGCCATATGGTAGCCTCATGTCCGC/ TAGCGTAAAAGGAGCAACATAG
Endogenous (qPCR)	LIN28 Endogenous (CDS)	AGCCATATGGTAGCCTCATGTCCGC/ TCAATTCTGTGCCTCCGGGAGCAGGGTAGG
House-Keeping Gene (qPCR)	GAPDH (1)	ACCACAGTCCATGCCATCAC/ TCCACCACCCTGTTGCTGTA
BBS5 pathogenic variants (Sanger Seq.)	BBS5 ex 4	ACCCACTGCTTACTGGCTTATCAGGAGACAGAATTGACCCTCT/ GAGGGGCAAACAACAGATGGCGCTTCAGTTTGGCCTCGTAA

Taqman probes

	Target	Assay ID
Pluripotency marker (qRT-PCR)	POU5F1/OCT4	Thermo Fisher Scientific Hs04260367_g1
Pluripotency marker (qRT-PCR)	NANOG	Thermo Fisher Scientific Hs04260366_g1
Pluripotency marker (qRT-PCR)	SOX2	Thermo Fisher Scientific Hs01053049_s1
Pluripotency marker (qRT-PCR)	TDGF1	Thermo Fisher Scientific Hs02339497_g1
Pluripotency marker (qRT-PCR)	DNMT3B	Thermo Fisher Scientific Hs00171876_m1
Pluripotency marker (qRT-PCR)	GARB3	Thermo Fisher Scientific Hs00241459_m1
Pluripotency marker (qRT-PCR)	GDF3	Thermo Fisher Scientific Hs00220998_m1
House-Keeping Gene (qRT-PCR)	GAPDH	Thermo Fisher Scientific Hs99999905_m1

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article

Acknowledgments

We thank Eva Pihl, Pia Hougaard and Pia Skovgaard for technical support and Jette Bune Rasmussen for assistance with generating the figures. The study was supported by grants from Rigshospitalet (R76-A2852) and Velux foundation (VELUX32700).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101594.

References

Forsythe, E., Beales, P.L., 2013. Bardet–Biedl syndrome. Eur. J. Hum. Genet. 21, 8–13. https://doi.org/10.1038/ejhg.2012.115.

Hjortshøj, T.D., Grønskov, K., Philp, A.R., Nishimura, D.Y., Riise, R., Sheffield, V.C., Rosenberg, T., Brøndum-Nielsen, K., 2010. Bardet-Biedl syndrome in Denmark-report of 13 novel sequence variations in six genes. Hum. Mutat. 31, 429–436. https://doi.org/10.1002/humu.21204.

Mourão, A., Christensen, S.T., Lorentzen, E., 2016. The intraflagellar transport machinery in ciliary signaling. Curr. Opin. Struct. Biol. 41, 98–108. https://doi.org/10.1016/j.sbi.2016.06.009.

Nachury, M.V., Loktev, A.V., Zhang, Q., Westlake, C.J., Peränen, J., Merdes, A., Slusarski, D.C., Scheller, R.H., Bazan, J.F., Sheffield, V.C., Jackson, P.K., 2007. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. Cell 129, 1201–1213. https://doi.org/10.1016/j.cell.2007.03.053.

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. https://doi.org/10.1038/nmeth.1591.