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Lab resource: Stem Cell Line

Characterization of the first induced pluripotent stem cell line generated from a patient with autosomal dominant catecholaminergic polymorphic ventricular tachycardia due to a heterozygous mutation in cardiac calsequestrin-2

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A B S T R A C T

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmia syndrome characterized by adrenaline induced ventricular tachycardia. The primary genetic aetiologies underlying CPVT are either autosomal dominant or autosomal recessive inheritance, resulting from heterozygous mutations in cardiac ryanodine receptor (*RYR2*) and homozygous mutations in cardiac calsequestrin-2 (*CASQ2*), respectively. Recently, a large family with autosomal dominant CPVT due to a heterozygous mutation in *CASQ2*, p.Lys180Arg, was reported. This resource is the first induced pluripotent stem cell line generated from a patient with autosomal dominant CPVT due to a heterozygous mutation in *CASQ2*. Induced pluripotent stem cells were generated from the whole blood of a 40-year-old woman with severe CPVT who is heterozygous for the p.Lys180Arg *CASQ2* mutation. Induced pluripotent stem cell (iPSC) characterization confirmed expression of pluripotency makers, trilineage differentiation potential, and the absence of exogenous pluripotency vector expression.

Resource table

Unique stem cell line identifier	MCCIi005-A-1
Alternative name(s) of stem cell line	BFC4
Institution	Centenary Institute
Contact information of distributor	Christopher Semsarian; c.semsarian@centenary.org.au
Type of cell line	Induced pluripotent stem cell
Origin	Human
Additional origin info	Age: 40 Sex: female Ethnicity: North-west European
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Transgene free (episomal vectors)
Genetic Modification	NA
Type of Modification	NA
Associated disease	Catecholaminergic Polymorphic Ventricular Tachycardia
Gene/locus	*CASQ2* p.Lys180Arg, c.539A > G, Chr1: 116275589 (on Assembly GRCh37)
Method of modification	NA
Name of transgene or resistance	NA
Inducible/constitutive system	NA
Date archived/stock date	2017
Cell line repository/bank	NA
Ethical approval	Patient informed consent obtained, and ethics review board-competent authority approval obtained through the Ethics Review Committee, Sydney Local Health District. Protocol No X13–0069 and HREC/13/RPAH/93

Resource utility

The pathophysiology underlying catecholaminergic polymorphic ventricular tachycardia (CPVT) is poorly understood. The need for functional studies is highlighted by the recent identification of autosomal dominant inheritance due to a heterozygous missense mutation which segregates with disease in a large Australian family. This iPSC line provides an unprecedented opportunity to understand disease pathophysiology and onset of life-threatening arrhythmias.

Resource details

CPVT is an arrhythmia syndrome characterized by adrenergically

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stimulated ventricular tachycardia, which can progress to sudden cardiac death.(Kozlovski et al., 2014) Diagnostic criteria include a structurally normal heart with normal baseline echocardiogram and development of exercise-induced polymorphic or bidirectional ventricular tachycardia.(Priori et al., 2013) The vast majority of CPVT cases are due to heterozygous mutations in cardiac ryanodine-2 receptor (*RYR2*), with rare cases resulting from homozygous cardiac calsequestrin-2 (*CASQ2*) mutations. The pathophysiology of CPVT is unclear. iPSCs offer a unique disease modelling platform. Recently, a large Australian family demonstrated CPVT with autosomal dominant inheritance due to a heterozygous missense mutation in *CASQ2*.(Gray et al., 2016) The iPSC line reported in this publication is the first to be generated from a patient with autosomal dominant *CASQ2* mediated disease. The iPSC line was generated from a 40 year-old-woman with a heterozygous mutation in *CASQ2* who became symptomatic at 14 years of age. Consistent with the lethal nature of the condition, the patient has an implantable cardioverter defibrillator and a family history of sudden cardiac death related to exercise events.

Peripheral blood mononuclear cells (PBMCs) were reprogrammed with episomal vectors carrying pluripotency factors Oct4, Sox2, Lin28, Klf4 and L-Myc. iPSC colonies were cultured and passaged in mTeSR™1. Cultures were free of mycoplasma contamination (Fig. 1G). Pluripotency marker expression was confirmed with quantitative PCR performed on iPSC mRNA (Fig. 1C), and immunofluorescence (Fig. 1A). Pluripotency potential was confirmed through trilineage differentiation (Fig. 1B). Cultural identity and purity was confirmed with identification of the rare *CASQ2* p.Lys180Arg mutation through Sanger sequencing (Fig. 1D, and short tandem repeat analysis at 16 unique sites performed on DNA isolated from iPSC culture and patients whole blood (archived with journal). Molecular karyotyping revealed a normal female karyotype with no aneuploidies detected (Fig. 1F). Characterization of the cell line is summarized in Table 1.

Materials and methods

Generation and maintenance of iPSC line

As previously reported,(Holliday et al., 2018) whole blood from the patient was collected, and PBMCs were isolated using the Erythroid Progenitor Reprogramming Kit (STEMCELL Technologies, Vancouver, CAN) according to manufacturer's protocol. In brief, PBMCs were isolated using the SepMate™ protocol and expanded in StemSpan II medium for 1 week. One million expanded PBMCs were nucleofected with 1 μL of each plasmid from the Epi5™ Episomal iPSC Reprogramming Kit (Thermo Fisher, MA, USA) using the CD34 + Nucleofector Kit with the Nucleofector I (Lonza Technologies, Basel, CHE) on program U-014 and plated onto Matrigel hESC-Qualified Matrix (Corning, NY, USA) coated plates. Approximately 15 days after transfection, single iPSC colonies appeared and were manually passaged and cultured and maintained in mTeSR™1 (STEMCELL Technologies). Cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

The LookOut™ Mycoplasma Kit (Sigma-Aldrich, MO, USA) was used, to determine lack of Mycoplasma contamination in cell cultures. A band at 481 bp represents an internal control whereas a band at 259 bp represents a positive result for the presence of mycoplasmaFig. 1G.

To determine loss of reprogramming vectors from cell cultures, DNA extracted from iPSCs was amplified for the presence of the Epi5 plasmid DNA. Primers used detect all vectors present in the Epi5 reprogramming Kit (Thermo Fisher) (Table 2). Amplified PCR products were run on a 2% agarose gel to confirm lack of PCR amplification in iPSC samplesFig. 1E).

Pluripotency marker expression and trilineage differentiation potential

For qualitative pluripotency analysis, iPSCs were plated on Matrigel coated glass coverslips and subsequently stained with antibodies against pluripotency markers: OCT4, SSEA4, SOX2 and TRA-1-60 (Table 2. For differentiation potential, iPSCs were differentiated into the 3 germ layers using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) and stained for lineage specific markers (Table 2). Mesoderm staining was performed on iPSC-cardiomyocytes differentiated using the STEMdiff™ Cardiomyocyte Differentiation Kit (STEMCELL Technologies). In brief, cells were washed with PBS, fixed in 4% paraformaldehyde for 15 min, permeabilised in 1% Saponin for 15 min and then blocked in 3% BSA for 30 min at room temperature. Cells were then incubated with primary antibodies (Table 2) for 3 h at room temperature in 3% BSA. After washing 3 times with PBS for 5 min, cells were incubated in secondary antibody (Table 2) for 1 h at room temperature in 3% BSA. Cells were washed 3 times in PBS for 5 min and then NucBlue™ (Thermo Fisher) was added for the last wash. Coverslips were mounted with ProLong™ Gold Antifade Mountant (Thermo Fisher) and imaged on the Leica SP5 confocalFig. 1A and 1B).

Quantitative PCR

Expression of pluripotency markers (*SOX2, OCT4* and *NANOG*) in iPSCs was compared to expression in PBMCs (Fig. 1C using qPCR. Briefly, RNA was extracted from samples using TRIzol™Reagent (Thermo Fisher) and was reverse transcribed using the SuperScript™ III Reverse Transcriptase kit (Thermo Fisher) according to manufacturer's protocol. qPCR was run on the Stratagene Mx3005P qPCR system (Aligent Technologies, CA, USA) using 500 ng of cDNA, primers supplied in Table 2 and the EXPRESS SYBR™ GreenER™ qPCR Supermix kit (Thermo Fisher). Ct values were normalized to GAPDH and analyzed as fold change from expression in PBMCs ($n = 3$).

Identification of pathogenic CASQ2 p.Lys180Arg mutation

Genomic DNA was isolated from iPSC at passage 12 using the NucleoBond CB DNA Isolation kit (Macherey-Nagel, Duren, DEU) according to manufacturer's protocol. DNA was subsequently PCR amplified using primers specific for exon 5 of the *CASQ2* gene (Table 2 and sent to Macrogen (Seoul, KOR) for Sanger sequencing. Chromatograms were analyzed using Sequencher 5.0.1 (Gene Codes Corporation, MI, USA) and confirmed the presence of the heterozygous *CASQ2* p.Lys180Arg c.539A > G mutation (Fig. 1D.

Short Tandem Repeat (STR) analysis

16 STR sites across the genome were amplified from the patient's blood DNA and iPSC cultures, with an untagged forward primer and a 6-FAM tagged reverse primer (Table 2). PCR products were sent to Macrogen for fragment analysis and then analyzed using Peak Scanner Software v1.0 (Thermo Fisher). Allele copy number was determined based on fragment size according to STRBase (National Institute of Standards and Technology, MD, US) and used to determine cell line purity.

Karyotype analysis

Genomic DNA from iPSC cultures was sent to Victorian Clinical Genetics Services (VCGS, VIC, AUS) for molecular karyotyping using the Illumina Infinium GSA-24 v1.0 at a resolution of 0.50 Mb (Fig. 1F).

Acknowledgements

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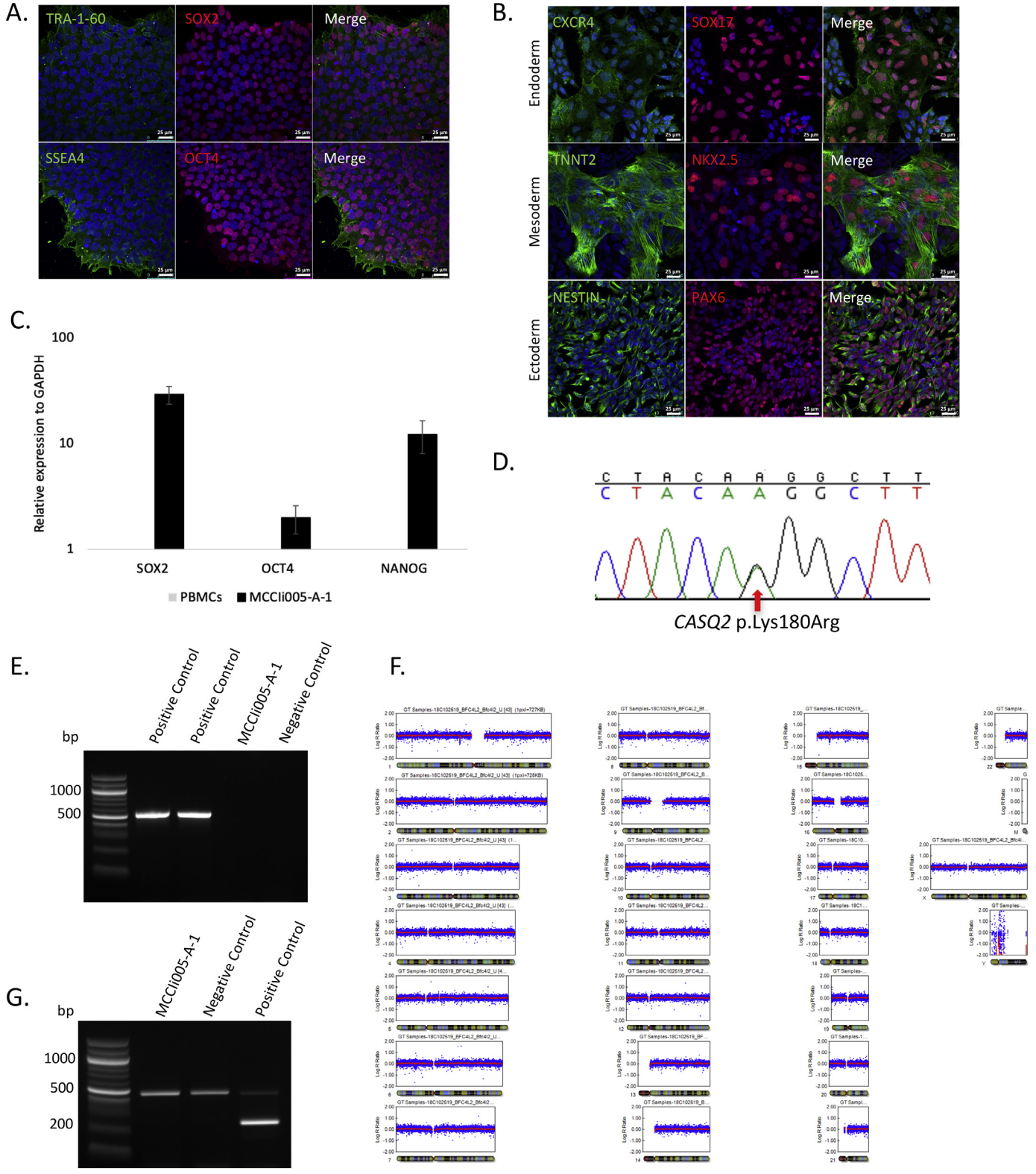

Fig. 1. A. Immunofluorescence imaging of iPSC for pluripotency markers (TRA-1-60, SOX2, SSEA4 and OCT4). Nuclei are counterstained with DAPI (blue). B. Immunofluorescence imaging of differentiated cells with endoderm (CXCR4 and SOX17), mesoderm (TNNT2 and NKX2.5) and ectoderm (NESTIN and PAX6) markers. Nuclei are counterstained with DAPI (blue). C. qPCR analysis of expression of pluripotency markers (SOX2, OCT4 and NANOG), normalised to GAPDH, in iPSC mRNA samples compared to PBMC mRNA (n = 3). D. Chromatogram showing presence of heterozygous CASQ2 p.Lys180Arg c.539A > G mutation in iPSC line. E. PCR demonstrating lack of amplification of episomal reprogramming vectors in iPSC DNA. Positive control: episomal reprogramming vectors. Negative control: untransfected DNA. F. Molecular karyotype of cell line demonstrating genomic integrity. Data is presented as the Log R Ratio for each chromosome. G. Mycoplasma testing of cell culture supernatant by PCR kit shows no presence of contamination in iPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the line shows normal morphology	Not shown but available with author
Phenotype	Qualitative analysis (Immunocytochemistry)	Presence of pluripotency markers (SOX2, TRA-1-60, OCT4 and SSEA4) confirmed by confocal imaging	Fig. 1, panel A Scale bar: 25 μm
	Quantitative analysis (RT-qPCR)	Expression of SOX2, OCT4 and NANOG	Fig. 1, panel C
Genotype	Karyotype (molecular) at resolution of 0.5 Mb	Normal female karyotype: (1–22,X)x2	Fig. 1, panel F
Identity	Microsatellite PCR (mPCR)	Not done	Not done
	STR analysis	16 STR sites tested with 100% match	Submitted in archive with journal
Mutation analysis	Sequencing	Heterozygous *CASQ2* p.Lys180Arg mutation present	Fig. 1, panel D
	Southern Blot OR WGS	Not done	Not done
Microbiology and virology	Mycoplasma	Mycoplasma by PCR testing was negative	Fig. 1, panel G
Differentiation potential	Directed differentiation	Presence of lineage specific markers (Endoderm: SOX17, CXCR4; Mesoderm: TNNT2, NKX2.5; Ectoderm: PAX6, NESTIN) confirmed by confocal imaging	Fig. 1, panel B Scale bar: 25 μm
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Not done	Not done
Genotype additional info	Blood group genotyping	Not done	Not done
	HLA tissue typing	Not done	Not done

Table 2
Reagents details.

Antibodies used for immunocytochemistry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Rabbit anti-OCT4	1:400	Cell Signaling Technology Cat#2750; RRID: AB_823583
Pluripotency marker	Rabbit anti-SOX2	1:400	Cell Signaling Technology Cat#3579; RRID: AB_2195767
Pluripotency marker	Mouse anti-SSEA4	1:500	Cell Signaling Technology Cat#4755; RRID: AB_1264259
Pluripotency marker	Mouse anti-TRA-1-60(S)	1:500	Cell Signaling Technology Cat#4746; RRID: AB_2119059
Endoderm marker	Mouse anti-CXCR4	1:100	R&D Systems Cat#AF1924; RRID: AB_355060
Endoderm marker	Mouse anti-SOX17	1:1000	Cell Signaling Technology Cat#81778; RRID: AB_2650582
Mesoderm marker	Mouse anti-TNNT2	1:500	Thermo Fisher Scientific Cat#A25969
Mesoderm marker	Mouse anti-NKX2.5	1:500	Thermo Fisher Scientific Cat#A25974
Ectoderm marker	Mouse anti-NESTIN	1:100	R&D Systems Cat#MAB1259; RRID: AB_2251304
Ectoderm marker	Rabbit anti-PAX6	1:40	Invitrogen Cat#42–6600; RRID: AB_2533534
Secondary for mouse antibodies	Alexa Fluor 488 Goat anti-mouse IgG	1:250	Thermo Fisher Scientific Cat#A11001; RRID: AB_2534069
Secondary for rabbit antibodies	Alexa Fluor 594 Goat anti-rabbit IgG	1:250	Thermo Fisher Scientific Cat#A11012; RRID: AB_141359
Primers			
	Target	Forward/Reverse primer (5′-3′)	
CASQ2 genotyping	Human *CASQ2* exon 5	GGTTTGGAGTTTTACGCGTCTT/AAGGGAGGATGGTTAATGTTGC	
Pluripotency qPCR	*SOX2*	AACCAGCGCATGGACAGTTA/ GACTTGACCACCGAACCCAT	
Pluripotency qPCR	*OCT4*	CCCCAGGGCCCCATTTTGGTACC/ ACCTCAGTTTGAATGCATGGGAGAGC	
Pluripotency qPCR	*NANOG*	AATGGTGTGACGCAGGGATG/ TGCACCAGGTCTGAGTGTTC	
Pluripotency qPCR	*GAPDH*	ACCACAGTCCATGCCATCAC/ TCCACCACCCTGTTGCTGTA	
Epi5 Plasmids	EBNA-1	TTCCACGAGGGTAGTGAACC/TCGGGGGTGTTAGAGACAAC	
STR Analysis	D5S818	GGTGATTTTCCTCTTTGGTATCC/(6-FAM)AGCCACAGTTTACAACATTTGTATCT	
STR Analysis	D13S317	ACAGAAGTCTGGGATGTGGA/(6-FAM)GCCCAAAAAGACAGACAGAA	
STR Analysis	D7S820	TGTCATAGTTTAGAACGAACTAACG/(6-FAM)CTGAGGTATCAAAAACTCAGAGG	
STR Analysis	D16S539	GATCCCAAGCTCTTCCTCTT/(6-FAM)ACGTTTGTGTGTGCATCTGT	
STR Analysis	vWA	GCCCTAGTGGATGATAAGAATAATCAGTATGTG/(6-FAM)GGACAGATGATAAATACATAGGATGGATGG	
STR Analysis	TH01	CAGCTGCCCTAGTCAGCAC/(6-FAM)GCTTCCGAGTGCAGGTCACA	
STR Analysis	Amelogenin	CCCTGGGCTCTGTAAAGAATAGTG/(6-FAM)ATCAGAGCTTAAACTGGGAAGCTG	
STR Analysis	TPOX	ACTGGCACAGAACAGGCACTTAGG/(6-FAM)GGAGGAACTGGGAACCACACAGGT	
STR Analysis	CSF1PO	AACCTGAGTCTGCCAAGGACTAGC/(6-FAM)TTCCACACACCACTGGCCATCTTC	
STR Analysis	D8S1179	TTTTTGTATTTCATGTGTACATTCG/(6-FAM)CGTAGCTATAATTAGTTCATTTTCA	
STR Analysis	D21S11	ATATGTGAGTCAATTCCCCAAG/(6-FAM)TGTATTAGTCAATGTTCTCCAG	
STR Analysis	D3S1358	ACTGCAGTCCAATCTGGGT/(6-FAM)ATGAAATCAACAGAGGCTTG	
STR Analysis	D18S51	GAGCCATGTTCATGCCACTG/(6-FAM)CAAACCCGACTACCAGCAAC	
STR Analysis	FGA	GCCCCATAGGTTTTGAACTCA/(6-FAM)TGATTTGTCTGTAATTGCCAGC	
STR Analysis	Penta E	ATTACCAACATGAAAGGGTACCAATA/(6-FAM)TGGGTTATTAATTGAGAAAACTCCTTACAATTT	
STR Analysis	Penta D	GAAGGTCGAAGCTGAAGTG/(6-FAM)ATTAGAATTCTTTAATCTGGACACAAG	

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