

Characterization of Human iPSC Line, SCTi003-A-3, ABCA4 Knockout

Catalog # 200-0992
Lot # 2505412010

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Product Information

Product	Human iPSC Line, SCTi003-A-3, ABCA4 Knockout
Catalog #	200-0992
Lot #	2505412010
Format	~1 million viable cells per vial
Date Viald	2025-05-13
Country of Manufacture	US
Stability, Storage, and Use Information	<p>Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.</p> <p>Product is derived from cells or tissues that are collected using consent forms and protocols approved by either an Institutional Review Board, the Food and Drug Administration, the U.S. Department of Health and Human Services, and/or an equivalent regulatory authority.</p> <p>FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.</p> <p>NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.</p>

Cell Line Information

Cell Line Name	SCTi003-A-3
Parent Material	SCTi003-A is a parent cell line
Gene Edit	ABCA4 knockout via CRISPR-Cas9–mediated frameshift mutation
Cell Type	Human Induced Pluripotent Stem Cell (hiPSC)
Passage Number of Cell Banks*	<p>Master Cell Bank: Passage 34 Commercial Cell Bank: Passage 37</p> <p>*This vial is from a SCTi003-A-3 commercial cell bank and was cultured for 36 passages prior to cryopreservation. +1 is added to the passage number on the vial to best represent the overall passage number of the cells at thaw.</p>
Source Cell Tissue	Blood
Source Cell Type	Peripheral Blood Mononuclear Cell (PBMC); $\alpha\beta$ T Cell
Reprogramming Vector	Non-Integrating

Recommended Culture Conditions

Maintenance Medium	mTeSR™ Plus (Cat # 100-0276)
Culture Type	Adherent
Supplement	Not Required
Substrate	Corning® Matrigel® hESC-Qualified Matrix
Dissociation Reagent	ReLeSR™ (Cat # 100-0484)
Dissociation Method	Non-enzymatic aggregate dissociation
Split Ratio	1:20 - 1:60 every 6 - 8 days
Incubator Atmosphere	37°C, 5% CO ₂ , and 95% humidity
Cryopreservation Reagent	CryoStor® CS10 (Cat # 07930/100-1061)
Thaw Recommendation	After thaw, pellet cells and resuspend in 1 mL mTeSR™ Plus. Aliquot into a pre-prepared six-well plate at six different densities: 150 µL, 100 µL, 75 µL, 50 µL, 25 µL, and 15 µL. Select the well with optimal colony density for passaging at Day 6 - 8.

Culture conditions are reflective of how the cell line was maintained prior to cryopreservation.

Donor Information

Age[†]	48	
Sex[‡]	Female	
Ethnicity and/or Race[†]	Caucasian	
Ancestry[‡]	0% African 100% European	0% East Asian 0% South Asian
Diagnosis[†]	Clinically unaffected at donation	
Height[‡]	168 cm	
Weight[‡]	62.1 kg	
BMI[‡]	22.1 kg/m ²	
Blood Type[‡]	B-	
Tobacco Use[†]	Non-smoker	
HLA Haplotype[‡]	HLA Class I: A*24:02:01G, 26:01:01G B*07:02:01G, - C*07:02:01G, - E*01:01:01G, - F*01:01:01G, 01:04 G*01:01:02G, 01:04:01G	HLA Class II: DRB1*15:01:01G, - DRB3*, - DRB4*, - DQA1*01:02:01G, - DQB1*06:02:01G, - DPA1*01:03:01G, - DPB1*02:01:02G, 04:01:01G

[†] Self-declared

[‡] Calculated

Gene Editing Summary

Gene	<i>ABCA4</i>
Edit Method	CRISPR-Cas9-mediated knockout (frameshift indels)
Parental Genotype	<i>ABCA4</i> wild-type
Edited Genotype	<i>ABCA4</i> knockout
Zygosity	Compound heterozygous frameshift (biallelic knockout)
Transcript ID	NM_000350.3
cDNA Change	c.209dupT c.207delG
Protein Change	p.Gln71fs p.Trp69fs

Overview:

The SCTi003-A-3 iPSC line was derived from the parental SCTi003-A line, which originated from a healthy female donor with a wild-type *ABCA4* genotype. Using CRISPR-Cas9 genome editing, two independent frameshift mutations were introduced in exon 3 of the *ABCA4* gene (NM_000350.3) to generate a biallelic knockout.

On allele 1, a single-base duplication at c.209dupT changes the reading frame beginning at codon 71, producing a frameshift mutation (p.Gln71fs). On allele 2, a single-base deletion at c.207delG alters the reading frame beginning at codon 69, resulting in a second frameshift mutation (p.Trp69fs).

Both frameshift mutations are present in trans, resulting in a compound heterozygous *ABCA4* knockout. Each mutation is predicted to disrupt *ABCA4* function, and together they result in loss of functional *ABCA4* protein. This engineered model provides a genetically defined system for investigating the role of *ABCA4* loss-of-function in retinal biology and for evaluating candidate therapeutic compounds.

DNA and protein alignments of the parental and edited alleles are shown in Figure 1.

Gene Editing Summary (cont.)

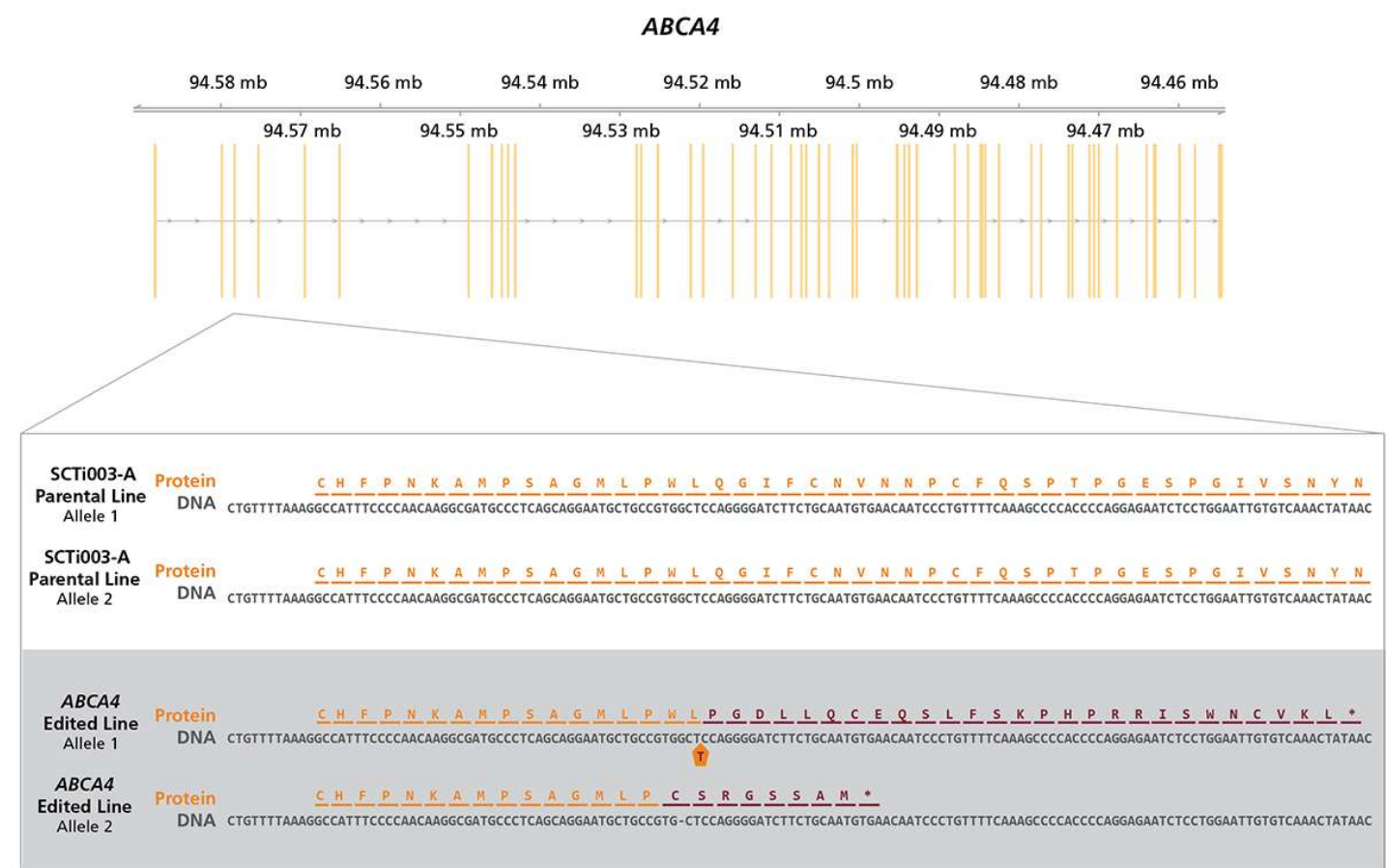


Figure 1. Gene editing of the *ABCA4* locus in the SCTi003-A-3 iPSC line

A schematic of the *ABCA4* gene structure is shown at the top of the figure, with exons (orange boxes) and introns (connecting lines) illustrated. CRISPR-Cas9 genome editing was used to generate a biallelic knockout of the *ABCA4* gene (NM_000350.3) in the parental SCTi003-A iPSC line (*ABCA4* wild-type genotype). Two distinct frameshift indels were introduced in exon 3: allele 1 contains a single-nucleotide duplication (c.209dupT), predicted to cause a frameshift starting at codon 71 (p.Gln71fs), and allele 2 contains a single-nucleotide deletion (c.207delG), predicted to cause a frameshift starting at codon 69 (p.Trp69fs). Both mutations are expected to introduce premature stop codons and abolish *ABCA4* function. The figure shows the DNA and protein sequences for both alleles of the parental and edited lines, with the edited bases highlighted. The DNA sequences are shown in the GRCh38 reference genome orientation for *ABCA4*, which is located on the negative strand of chromosome 1. The gene edit was confirmed by whole-exome and whole-genome sequencing at ~50× coverage.

Results Summary

Assessment	Analytical Method	Acceptance Criteria	Result
Viability ^{CCB}	Viability assessment performed on thawed cells using the NucleoCounter® NC-250™ by ChemoMetec	≥ 60% viable	Pass
Recovery ^{CCB}	Cells recovered using specified thaw and culture recommendations	Recoverable attachment 24 hr. after plating and cells grow to confluency	Pass
Cell Line Identity ^{CCB}	STR amplification performed using the Powerplex 16 HS System by Promega	Match	Pass
Sterility ^{CCB}	Presence or absence of bacterial and fungal organisms by incubation in TSB and FTB for 14 days	Negative	Pass
Mycoplasma ^{CCB}	Presence or absence of mycoplasma using the EZ-PCR™ Mycoplasma Detection Kit by Sartorius	Negative	Pass
Karyotype ^{CCB}	GTL Banding performed on 20 metaphase cells	Normal	Pass
20q Amplification ^{MCB}	Fluorescence in situ hybridization (FISH)	Negative	Pass
Copy Number Variants ^{CCB}	Genomic DNA analyzed using Illumina Global Diversity Array with Cytogenetics-8 (GDACyto)	No Specification	Reported
Gene Edit Verification ^{MCB}	IGV analysis of whole exome and whole genome sequencing data	On-target edit confirmed	Pass
Genetic Variants ^{MCB}	Whole exome and whole genome sequencing data analyzed using ClinVar	No Specification	Reported
TP53 and BCOR Status ^{MCB}	Whole exome and whole genome sequencing data analyzed using ClinVar	No Specification	Reported
Undifferentiated Status ^{CCB}	Three-passage assay and flow cytometry for undifferentiated cell markers	OCT4+, TRA-1-60+ ≥ 80%	Pass
Pluripotency ^{MCB}	Flow cytometry performed on cells differentiated into endoderm, mesoderm, and ectoderm germ layers	Endoderm: CXCR4+, SOX17+ ≥ 70% Mesoderm: T+, NCAM+ ≥ 70% Ectoderm: PAX6+, NESTIN+ ≥ 70%	Pass

^{CCB}Assessment performed on the Commercial Cell Bank

^{WCB}Assessment performed on the Working Cell Bank

^{MCB}Assessment performed on the Master Cell Bank

^{PMB}Assessment performed prior to the Master Cell Bank

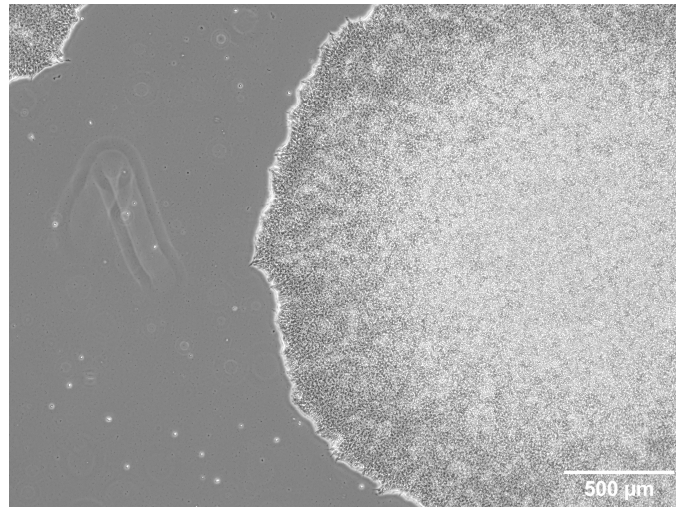
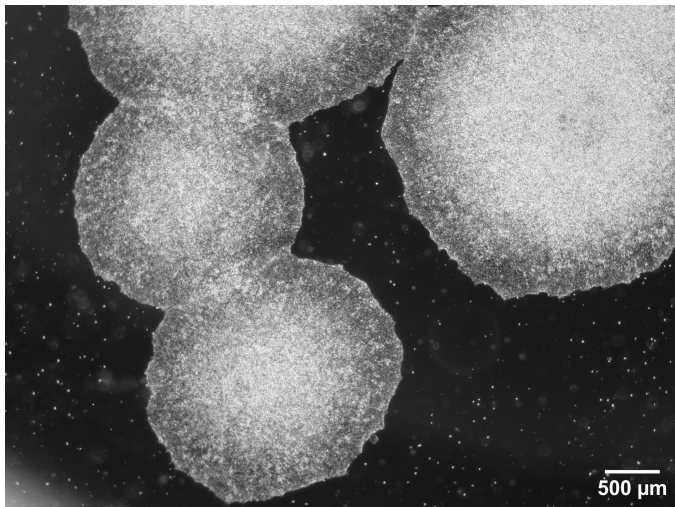
X *um* 2025-08-20

Approved by Initial & Date

Morphology Report

Sample	SCTi003-A-3 Lot # 2505412010
Submitted Passage #	40
Analysis Date	2025-06-24

Results:



Interpretation:

Sample demonstrated round colonies containing tightly packed cells with a high nucleus-to-cytoplasm ratio and prominent nucleoli. Colony centers were dense and appeared bright under a phase contrast microscope. This morphology is consistent with the undifferentiated state.

Assay Description:

Sample is thawed as described in the Product Information Sheet and cultured in mTeSR™ Plus (#100-0276) on Corning® Matrigel® hESC-Qualified Matrix for three passages using ReLeSR™ (#100-0484). Images are captured at 20X and 40X magnification on Day 7 of Passage 3.

Viability and Recovery Report

Sample	SCTi003-A-3 Lot # 2505412010
Viability Platform	NucleoCounter® NC-250™
Viability Protocol	Viability and Cell Count - A100 and B Assay
Viability Analysis Date	2025-05-30
Recovery Completion Date	2025-06-05

Results:

Viability	90.4%
Recovery after 24h	<input checked="" type="checkbox"/>
Cells Grow to Confluence	<input checked="" type="checkbox"/>

Assay Description:

Viability: iPSC aggregates are analyzed at thaw using the NucleoCounter® NC-250™ Viability and Cell Count - A100 and B Assay. Cell aggregates are disaggregated, singularized, and stained with DAPI. Viability % represents the mean of two counts.

Recovery: Sample is thawed and recovered as described in the Product Information Sheet. At 24 h after thaw, the culture is assessed for the number of adherent cellular aggregates. Cells are expanded until the culture reaches an optimal density consisting of large, multilayered colonies that have begun to merge.

Cell Line Identity Report

	SCTi003-A-3 Lot # 2505412010	SCTi003-A-3 Master Cell Bank
Samples Received Date	2025-05-20	2024-08-07
STR Amplification Date	2025-06-05	2024-08-21

Short Tandem Repeat (STR) Analysis

Sample Name	SCTi003-A-3 Lot # 2505412010	SCTi003-A-3 Master Cell Bank
CTR No. †	107574	103364
FGA	26, 26	26, 26
TPOX	8, 8	8, 8
D8S1179	12, 13	12, 13
vWA	17, 17	17, 17
Amelogenin	X, X	X, X
Penta_D	12, 14	12, 14
CSF1PO	10, 10	10, 10
D16S539	11, 11	11, 11
D7S820	9, 12	9, 12
D13S317	11, 11	11, 11
D5S818	11, 12	11, 12
Penta_E	8, 17	8, 17
D18S51	15, 17	15, 17
D21S11	27, 29	27, 29
TH01	9.3, 9.3	9.3, 9.3
D3S1358	17, 17	17, 17
Allelic Polymorphisms	22	22
Matches*	103364	107574
Comments		

†CTR No.: Characterization Test Request Number; also known as a laboratory accessioning number.

*Note: The STR profile of the following sample is a 100% match for the given sample/samples.

Cell Line Identity Report (cont.)

Assay Description:

STR Analysis is performed using the PowerPlex 16 HS System by Promega™. Results are reported as 13 CODIS STR markers, Amelogenin for sex determination and two low-stutter, highly discriminating pentanucleotide STR markers.

Results:

The genotypic profiles comprise a range of 22 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation:

The concentration of DNA required to achieve an acceptable STR genotype (signal/noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. These results suggest that the cells submitted correspond to the cell lines as named and were not contaminated with any other human cells or a significant amount of mouse feeder layer cells.

Sensitivity:

Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2 - 4%.

Sterility Report

Collection Date	2025-05-20
Approval Date	2025-06-06

Diagnostic Summary

Test	Colony	Tested	+	+/-	?	PDG
All results NEGATIVE						

+ = Positive; +/- = Equivocal; ? = Indeterminate; PDG = Pending

Bacteriology – Sterility Test – Broth Cultures: 2 Samples

	SCTi003-A-3 Lot # 2505412010 Cryovial 1	SCTi003-A-3 Lot # 2505412010 Cryovial 2	SCTi003-A-3 Lot # 2505412010 Cryovial 3
Sterility Test (TSB, FTM)	-	-	-

Remarks:

- = Negative/No Growth as determined by culture conditions; + = Positive/Growth Present
NE = Not Evaluated: Samples evaluated on scheduled business days; NI = Not Interpreted: Culture could not be interpreted due to overgrowth of Proteus; NT = Not Tested; TSB = Tryptic soy broth; FTM = Fluid thioglycollate media.

Mycoplasma Report

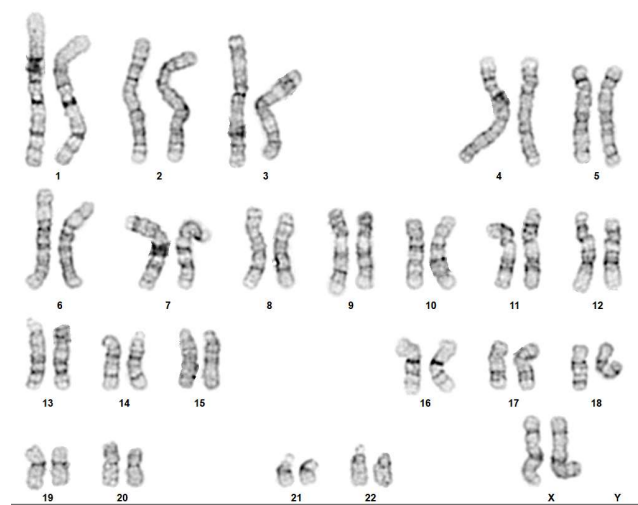
Date Reported	2025-05-23
Assay Description	Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit (Sartorius).

Sample Name	Result	Interpretation
SCTi003-A-3 Lot # 2505412010	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma
Positive (+) Control	Positive	
Negative (-) Control	Negative	

Chromosome Analysis Report

GTL-Banded Karyotype Analysis

Date Reported	2025-05-26
Sample	SCTi003-A-3 Lot # 2505412010
Cell Line Sex	Female
Submitted Passage #	37
Date of Sample	2025-05-20
Specimen	Human iPSC
Results	46,XX



Cell	5
Slide	3
Slide Type	Karyotype
Total Counted	20
Total Analyzed	20
Total Karyogrammed	20
Band Resolution	550

Interpretation:

This is a normal karyotype; no clonal abnormalities were observed at the stated band level of resolution.

Limitations:

This assay allows for microscopic visualization of numerical and structural chromosome abnormalities. The size of structural abnormality that can be detected is > 3 - 10Mb, dependent upon the G-band resolution obtained from this specimen. For the purposes of this report, band level is defined as the number of G-bands per haploid genome. It is documented here as “band level”, i.e., the range of bands determined from the twenty karyograms in this assay. Detection of heterogeneity of clonal cell populations in this specimen (i.e., mosaicism) is limited by the number of metaphase cells examined, documented here as “# of cells counted”.

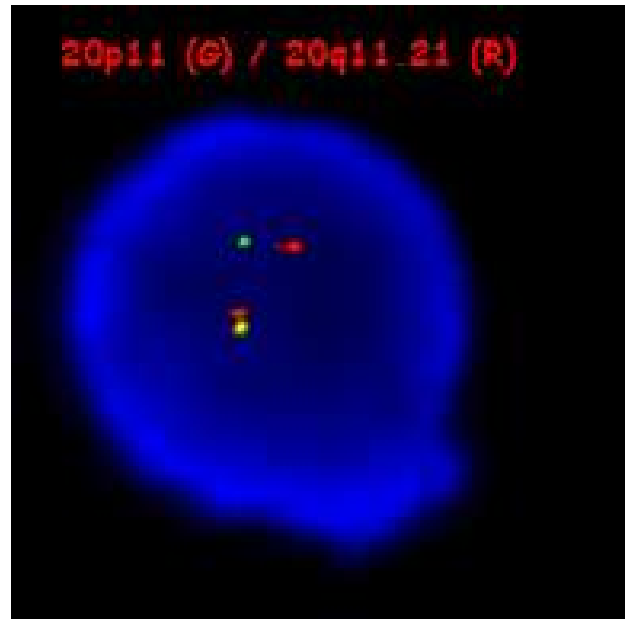
20q Status Report

Fluorescence In-Situ Hybridization (FISH) Analysis

Date Reported	2024-08-19
Sample	SCTi003-A-3 Master Cell Bank
Cell Line Sex	Female
Submitted Passage #	34
Date of Sample	2024-08-07
Specimen	Human iPSC

Probe	# of cells with 2G1R pattern	# of cells with 2G2R pattern	# of cells with 2G3R pattern	# of cells with 1G2R pattern
20p11 (G) / BCL2L1 (R)	3 / 200 (1.5%)	193 / 200 (96.5%)	3 / 200 (1.5%)	1 / 200 (0.5%)
Cutoff	4%	N/A	5%	4%

Probe: 20p11 (G) / 20q11.21 (R)



Interpretation:
There is no evidence for aneusomy of chromosome 20. Two probe signals were observed in 96.5% of two hundred interphase cells examined for the 20p11.21 and 20q11.21 (BCL2L1) regions.

Copy Number Variants (CNV) Report

Microarray Analysis

Date Reported	2025-07-22
Sample	SCTi003-A-3 Lot # 2505412010
Cell Line Sex	Female
Submitted Passage #	37
Date of Sample	2025-05-20
Specimen	Human iPSC

Microarray Results arr[GRCh37] 7q34(142023396_142475348)x1,14q11.2(22327490_22789110)x1

Call Table:

Chr	Cytoband	Event (% Mosaic)	Estimated Copy Number	Start	End	Length (Base Pairs)	Gene Count
7	7p14.1	Homozygous Loss	0	38,311,831	38,338,251	26,421	1
7	7q34	Loss	1	142,023,396	142,475,348	451,953	2
14	14q11.2	Loss	1	22,327,490	22,789,110	461,621	0
14	14q11.2	Homozygous Loss	0	22,790,594	22,961,867	171,274	1
14	14q32.33	Homozygous Loss	0	106,067,118	106,141,427	74,310	3
14	14q32.33	Loss	1	106,150,580	106,236,278	85,699	0

Interpretation:

There were 2 reportable copy number changes as well as 0 reportable regions of LOH identified:

- A 0.452Mb loss on chromosome 7 was observed.
- A 0.462Mb loss on chromosome 14 was observed.

Copy Number Variants (CNV) Report (cont.)

Specifications:

- Platform: Illumina: Global Diversity Array with Cytogenetics-8 (GDACyto)
- Marker coverage: 1,825,277 spanning whole human genome
- Analysis software: Bionano: Via™ Software
- Array design, genomic position, genes and chromosome banding are based on genome build GRCh37/hg19.
- Aberrant copy number genomic regions are identified by log R ratio (LRR) and B allele frequency (BAF). LRR is the log ratio of observed probe intensity to expected intensity, deviations from zero are evidence for copy number change. BAF is the proportion of hybridized sample that carries the B allele: 0.0, 0.5, and 1.0 are expected for each locus in a normal sample. Deviations from this expectation are indicative of aberrant copy number.
- Quality assurance monitors: 1) Call Rate; 2) Confidence Threshold; 3) LogRDev; 4) Illumina sample dependent/independent QC measures.
- Reportable copy number changes are gains or losses greater than 400kb. Reportable regions of LOH are greater than 5Mb. See Interpretation for copy number changes and regions of LOH that meet these criteria. See Call Table for all copy number changes identified by the analysis software. If mosaicism is detected, the approximate percentage of mosaicism is listed in the 'Event (% Mosaic)' column.
- Copy number changes and regions of LOH are reported at greater than 10% and 20% mosaicism respectively.
- The assay is currently validated for the detection of copy number losses greater than 20kb in size and copy number gains 50kb in size (smaller changes may be detected depending on gene content and probe number but will not be included in the Call Table). From validation studies, abnormalities present in a mosaic state are reliably detected if the mosaicism level (percentage of abnormal cells) is 20% or higher.
- Sample intensities were compared to standard cluster file intensities comprised of over 100 samples from Caucasian (CEU), Asian (CHB+JPT), and Yoruban (YRI) HapMap populations.

Limitations:

This assay will detect aneuploidy, deletions, and duplications of represented loci, and regions of loss/absence of heterozygosity (LOH), but will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and insertions), or point mutations. Based on the results of internal validation studies, abnormalities present in a mosaic state are reliably detected if the mosaicism level (percentage of abnormal cells) is 20% or higher. The failure to detect an alteration at any locus does not exclude all anomalies at that locus. Significance of the number of probes used to detect an aberration has not been determined and confirmational testing may be informative. Actual chromosomal localization of copy number change is not determined by this assay. Other mapping procedures are required for determining chromosomal localization.

Gene Edit Verification Report

Sample	SCTi003-A-3 Master Cell Bank
Whole Exome and Whole Genome Sequencing Report	2025-05-27

Parental Line (SCTi003-A, *ABCA4* wild-type) – chr1:94111531 Sequence (Reverse Complement):

AGGCCATTTCCCCAACAAGGCGATGCCCTCAGCAGGAATGCTGCCGTGGCT-CCAGGGGATCTTCTGCAATGTGA
ACAATCCCTGTTTTCAAAGCCCCACCC

Edited Line (SCTi003-A-3, *ABCA4* knockout) – chr1:94111531 Sequence (Reverse Complement):

AGGCCATTTCCCCAACAAGGCGATGCCCTCAGCAGGAATGCTGCCGTG-CTICCAGGGGATCTTCTGCAATGTGA
ACAATCCCTGTTTTCAAAGCCCCACCC

Edit: Two independent frameshift mutations were introduced in exon 3 of the *ABCA4* gene (NM_000350.3) using CRISPR-Cas9: c.209dupT (p.Gln71fs) and c.207delG (p.Trp69fs). These mutations occur on separate alleles, resulting in a compound heterozygous biallelic knockout. Both frameshifts disrupt the reading frame early in the coding sequence and are predicted to abolish *ABCA4* function.

Resulting Genotype: Compound heterozygous *ABCA4* knockout (c.209dupT, p.Gln71fs / c.207delG, p.Trp69fs).

Analysis Description:

Preprocessing:

Genomic DNA from the sample underwent whole-genome sequencing (WGS) using the NovaSeq X Plus System (Illumina), generating paired-end 150 bp reads at an average coverage of 50x across the genome. Prior to sequencing, DNA was randomly fragmented, end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified to generate the final sequencing libraries. To further enhance variant detection in coding regions, whole-exome capture was performed using the SureSelect Human All Exon V6 kit (Agilent Technologies), enriching for ~60 Mb of exonic and splice junction regions across approximately 20,000 human genes. Sequencing reads were aligned to the GRCh38 human reference genome, and single nucleotide variants (SNVs) and insertions/deletions (indels) were identified following GATK best practices (v4.6.1.0).

Gene Edit Verification:

Edit site coordinates were provided by the Genome Engineering & Stem Cell Center (GESC@MGI). The gene edit was verified by visual inspection using Integrative Genomics Viewer (IGV v2.18.0), comparing the parental and edited iPSC lines at the target site. A consensus sequence spanning ±50 bp around the edited locus was generated and aligned to the GRCh38 reference genome using BLAST to confirm on-target specificity and rule out off-target integration.

Genetic Variants Report

Sample	SCTi003-A-3 Master Cell Bank
Whole Exome and Whole Genome Sequencing Report Date	2025-05-27
ClinVar Version Date	2024-02-15

Pathogenic or Likely Pathogenic Variants:

Gene	ClinVar ID	Coordinates	Molecular Consequence	Nucleotide Change	Protein Change
ABCA4	99390	chr1:94010911	Missense variant	c.5603A>T	N1868I
AMPD1	18271	chr1:114693436	Nonsense	c.34C>T	Q12*
APOE	17864	chr19:44908684	Missense variant	c.388T>C	C112R
BCHE	13220	chr3:165773492	Missense variant	c.1699G>A	A567T
CTRC	8178	chr1:15445717	Missense variant	c.760C>T	R254W
ITPKB	1705897	chr1:226735804	Missense variant	c.1655C>A	P552Q
PRODH	4011	chr22:18918451	Missense variant	c.1292G>A	R323H
PTEN	810888	chr10:87863959	Missense variant	c.-511G>A	G4R
SLC29A3	568	chr10:71344255	Missense variant	c.347T>G	M116R
SPTA1	258954	chr1:158618068	Intron variant	c.6531-12C>T	
SPTA1	258948	chr1:158627717	Missense variant	c.5572C>G	L1858V
TYR	3778	chr11:89178528	Missense variant	c.575C>A	S192Y

Genetic Variants Report (cont.)

Analysis Description:

Sequencing and Analysis Workflow:

Genomic DNA from the sample underwent whole-genome sequencing (WGS) using the NovaSeq X Plus System (Illumina), generating paired-end 150 bp reads at an average coverage of 50x across the genome. Prior to sequencing, DNA was randomly fragmented, end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified to generate the final sequencing libraries. To further enhance variant detection in coding regions, whole-exome capture was performed using the SureSelect Human All Exon V6 kit (Agilent Technologies), enriching for ~60 Mb of exonic and splice junction regions across approximately 20,000 human genes. Sequencing reads were aligned to the GRCh38 human reference genome, and single nucleotide variants (SNVs) and insertions/deletions (indels) were identified following GATK best practices (v4.6.1.0).

Variant Filtering and Classification:

Single nucleotide variants (SNVs) and insertions/deletions (indels) were retained if they met the convolutional neural network (CNN) filtering threshold and were supported by a minimum of two sequencing reads. All retained variants were cross-referenced against ClinVar (version 2024-02), a publicly available database curated by the NCBI that links human genetic variants to associated phenotypes. Only variants classified by ClinVar as *pathogenic* or *likely pathogenic* were included in this report.

Disclaimer:

Pathogenicity classifications are based solely on ClinVar annotations and have not been independently validated by STEMCELL. Classifications reflect the state of knowledge at the time of reporting and may be subject to change as new evidence emerges.

TP53 and BCOR Status Report

Sample	SCTi003-A-3 Master Cell Bank
Whole Exome and Whole Genome Sequencing Report Date	2025-05-27
ClinVar Version Date	2024-02-15

Overview:

Gene	% Exon Covered by 15+ Reads	Average Exonic Read Depth	# Exonic or Impact Variants Detected
TP53	98.93%	106.56 (sd:75.96)	1
BCOR	100.00%	100.74 (sd:82.41)	2

TP53:

Locus	ClinVar ID	Nucleotide Change	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chr17:7676154	12351	c.215C>G	p.Pro72Arg	Missense variant	0	157	Germline homozygous

BCOR:

Locus	ClinVar ID	Nucleotide Change	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chrX:40073555	95767	c.1791C>T	p.His597His	Synonymous variant	61	93	Germline heterozygous
chrX:40074086	95764	c.1260T>C	p.Asp420Asp	Synonymous variant	114	99	Germline heterozygous

***TP53* and *BCOR* Status Report (cont.)**

Interpretation:

No pathogenic or likely pathogenic variants were identified in *TP53* and *BCOR*. No variants were identified in *TP53* that were previously reported as common recurring mutations in human pluripotent stem cell cultures by Merkle et al. (2017).

Analysis Description:

Sequencing and Analysis Workflow:

Genomic DNA from the sample underwent whole-genome sequencing (WGS) using the NovaSeq X Plus System (Illumina), generating paired-end 150 bp reads at an average coverage of 50x across the genome. Prior to sequencing, DNA was randomly fragmented, end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified to generate the final sequencing libraries. To further enhance variant detection in coding regions, whole-exome capture was performed using the SureSelect Human All Exon V6 kit (Agilent Technologies), enriching for ~60 Mb of exonic and splice junction regions across approximately 20,000 human genes. Sequencing reads were aligned to the GRCh38 human reference genome, and single nucleotide variants (SNVs) and insertions/deletions (indels) were identified following GATK best practices (v4.6.1.0).

Variant Filtering and Classification:

Single nucleotide variants (SNVs) and insertions/deletions (indels) were retained if they met the convolutional neural network (CNN) filtering threshold and were supported by a minimum of two sequencing reads. All retained variants were cross-referenced against ClinVar (version 2024-02), a publicly available database curated by the NCBI that links human genetic variants to associated phenotypes. All exonic variants in the *TP53* and *BCOR* genes that meet these criteria and result in amino acid changes are reported. For *TP53*, variants are further cross-referenced with those described by Merkle et al. (2017).

Disclaimer:

Pathogenicity classifications are based solely on ClinVar annotations and have not been independently validated by STEMCELL. Classifications reflect the state of knowledge at the time of reporting and may be subject to change as new evidence emerges.

Reference:

Merkle, FT. et al. (2017) Human pluripotent stem cells recurrently acquire and expand dominant negative P53 mutations. *Nature*. 545(7653):229-233.

Undifferentiated Status Report

Flow Cytometric Analysis

Sample	SCTi003-A-3 Lot # 2505412010
Submitted Passage #	40
Analysis Date	2025-06-24
# of Events Analyzed	10,000

Results:

Marker	Expression
OCT4	87.6%
TRA-1-60	97.0%

Interpretation:

Upon examination, a high percentage of cells exhibited OCT4 and TRA-1-60 markers of the undifferentiated status, indicative of a primarily undifferentiated cell culture.

Assay Description:

Sample is thawed and cultured for three consecutive passages, then singularized for undifferentiated marker expression analysis by flow cytometry at the end of passage 3. Results are analyzed using FlowJo™ software. Results are presented as the mean marker expression of two technical replicates.

Pluripotency Report

In Vitro Directed Trilineage Differentiation Analysis

Sample	SCTi003-A-3 Master Cell Bank
Submitted Passage #	35
Analysis Date	2025-01-23

Results:

Lineage	Marker	Expression
Endoderm	SOX17	94.0%
	CXCR4	98.4%
Mesoderm	BRACHYURY (T)	94.9%
	NCAM	90.4%
Ectoderm	PAX6	94.4%
	NESTIN	92.6%

Interpretation:

Following directed differentiation, expression was observed for markers specific to each lineage: endoderm, mesoderm, and ectoderm. This result is consistent with the pluripotent state.

Assay Description:

Sample undergoes directed differentiation using the STEMdiff™ Definitive Endoderm Kit (Cat # 05110), STEMdiff™ Mesoderm Induction Medium (Cat # 05221), and the STEMdiff™ SMADi Neural Induction Kit (Cat # 08581). Expression of lineage-specific markers is assessed by flow cytometry following five days of culture for endoderm and mesoderm lineages, and following seven days of culture for the ectoderm lineage. Results are reported as the percent of total cells with positive expression for each individual lineage-specific marker. Results are presented as the mean marker expression of two technical replicates.

