Characterization of Healthy Control Human iPSC Line, Female, SCTi003-A

Catalog # 200-0511
Lot # 2307406009

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Product Information

Product	Healthy Control Human iPSC Line, Female, SCTi003-A
Catalog #	200-0511
Lot #	2307406009
Format	~1 million viable cells per vial
Date Vialed	2023-07-06
Country of Manufacture	US
Stability, Storage, and Use Information	Product stable at -135°C or colder for 12 months from date of receipt. Short-term storage of cells (< 1 month) at -80°C is acceptable, but should be minimized to ensure maximum stability. Thawed samples must be used immediately. 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. FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS. NOT APPROVED FOR HUMAN OR VETERINARY USE IN VIVO.

Cell Line Information

Cell Line Name	SCTi003-A
Parent Material	SCTi003-A is a parent cell line
Cell Type	Human Induced Pluripotent Stem Cell (hiPSC)
Passage Number of Cell Banks*	**Master Cell Bank**: Passage 26 **Working Cell Bank**: Passage 29 **Commercial Cell Bank**: Passage 32 *This vial is from a SCTi003-A commercial cell bank and was cultured for 31 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.
Source Cell Tissue	Blood
Source Cell Type	Peripheral Blood Mononuclear Cell (PBMC); αβ 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	1:30 - 1:60 every 5 - 7 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 7.

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

Donor Information

Age†	48	
Sex‡	Female	
Diagnosis†	Clinically unaffected at donation	
Ethnicity and/or Race†	White	
Ancestry‡	0% African 78.2% European	0% East Asian 21.8% South Asian
Height‡	168 cm	
Weight‡	62.1 kg	
BMI‡	22.1 kg/m^2	
Blood Type‡	B-	
HLA Haplotype‡	**HLA Class I** A*24:02:01G, 26:01:01G B*07:02:01G, - C*07:02:01G, -	**HLA Class II** DRB1*15:01:01G, - DRB3*-, - DRB4*-, - DRB5*01:01:01G, - DQB1*06:02:01G, - DPB1*02:01:02G, 04:01:01G
ClinVar Analysis‡§	Five pathogenic or likely pathogenic variants in the following genes: AGXT, KLKB1, NQO1, RUNX1, and SLC12A3.	
Tobacco Use†	Non-smoker	

† Self-declared
‡ Calculated
§ Based on data from the 1000 Genomes Project, the average person has 18 pathogenic or likely pathogenic variants

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
Viral Screen[MCB]	Human Comprehensive CLEAR PCR Panel	Negative	Pass
Parental Cell Lineage[PMB]	Presence or absence of TCR Gene Rearrangements using the T cell clonality assay	No Specification	Reported
Residual Vector[PMB]	Genomic DNA analyzed by PCR	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
Ancestry[MCB]	Whole exome sequencing data analyzed using EthSeq	No Specification	Reported
Genetic Variants[MCB]	Whole exome sequencing data analyzed using ClinVar	No Specification	Reported
TP53 and BCOR Status[MCB]	Whole exome 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 using the STEMdiff™ Trilineage Differentiation Kit	**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

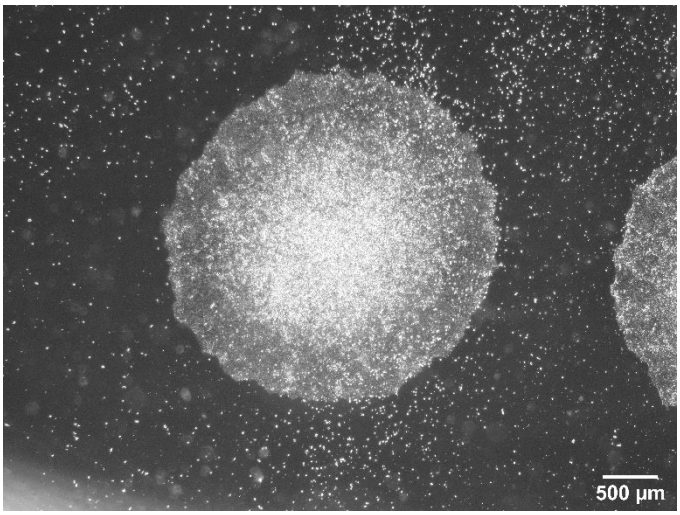
X vm 2024-06-05

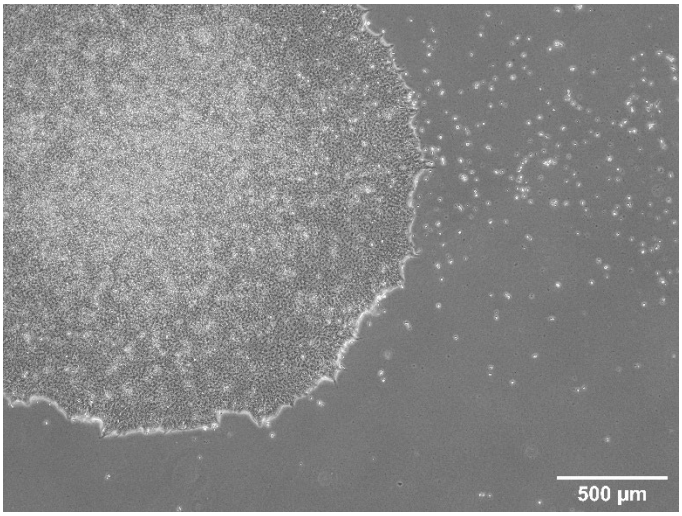
Approved by Initial & Date

Morphology Report

Sample	SCTi003-A Lot # 2307406009
Submitted Passage #	35
Analysis Date	2023-08-24

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 Lot # 2307406009
Viability Platform	NucleoCounter® NC-250™
Viability Protocol	Viability and Cell Count - A100 and B Assay
Viability Analysis Date	2023-07-27
Recovery Completion Date	2023-08-03

Results:

Viability	68.4%
Recovery after 24h	☒
Cells Grow to Confluence	☒

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 Lot # 2307406009	SCTi003-A Master Cell Bank
Samples Received Date	2023-08-09	2021-11-30
STR Amplification Date	2023-08-29	2021-12-20

Short Tandem Repeat (STR) Analysis

Sample Name	SCTi003-A Lot # 2307406009	SCTi003-A Master Cell Bank
CTR No. †	98230	89678
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*	89678	98230
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	2023-07-12
Approval Date	2023-08-02

Diagnostic Summary

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

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

Bacteriology – Sterility Test – Broth Cultures: 2 Samples

	SCTi003-A Lot # 2307406009 Cryovial 1	SCTi003-A Lot # 2307406009 Cryovial 2
Day 1 (TSB, FTM)	-	-
Day 2 (TSB, FTM)	NE	NE
Day 3 (TSB, FTM)	NE	NE
Day 4 (TSB, FTM)	-	-
Day 5 (TSB, FTM)	-	-
Day 6 (TSB, FTM)	-	-
Day 7 (TSB, FTM)	-	-
Day 8 (TSB, FTM)	-	-
Day 9 (TSB, FTM)	NE	NE
Day 10 (TSB, FTM)	NE	NE
Day 11 (TSB, FTM)	-	-
Day 12 (TSB, FTM)	-	-
Day 13 (TSB, FTM)	-	-
Day 14 (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	2023-08-22
Assay Description	Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit (Sartorius).

Sample Name	Result	Interpretation
SCTi003-A Lot # 2307406009	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma
Positive (+) Control	Positive	
Negative (-) Control	Negative	

Viral Screen Report

Collection Date	2021-12-10
Approval Date	2021-12-16

Molecular Diagnostics – Infectious Disease PCR
Human Comprehensive CLEAR Panel

	SCTi003-A Master Cell Bank
AAV2 (Adeno-Associated Virus 2)	-
BK Virus	-
Epstein-Barr Virus	-
Hantaan PCR	-
Hepatitis A Virus	-
Hepatitis B Virus	-
Hepatitis C Virus	-
Herpes Simplex Virus 1 PCR	-
Herpes Simplex Virus 2 PCR	-
Herpes Virus Type 6	-
Herpes Virus Type 7	-
Herpes Virus Type 8	-
HIV-1	-
HIV-2	-

	SCTi003-A Master Cell Bank
HPV-16	-
HPV-18	-
Human Adenovirus PCR	-
Human Cytomegalovirus	-
Human Foamy Virus	-
Human T-Lymphotropic Virus	-
John Cunningham Virus	-
LCMV PCR	-
Parvovirus B19	-
Sarbecovirus (SARs Virus) PCR	-
Seoul Virus PCR	-
C. bovis PCR	-
Mycoplasma Genus PCR	-

Remarks:
- = Negative; +/- = Equivocal; + = Positive; I = Inconclusive
An equivocal result indicates inconsistent amplification detected by real-time PCR.
Inconclusive indicates failure of control result.

Nucleic Acid Recovery Control (NRC)/Inhibition Control: A low copy exogenous nucleic acid was added to sample lysis prior to nucleic acid isolation to serve as both a control to monitor for nucleic acid recovery and PCR inhibition. An RNA NRC also monitors reverse transcription for RNA virus assays. Nucleic acid recovery and PCR inhibition is monitored by a PCR assay specific for the NRC template. Unless otherwise stated, control results passed for this order.

Any samples reported as equivocal or positive result in this report has been confirmed by re-extracting nucleic acid and repeating real-time PCR amplification to confirm the initial testing result.

Parent Cell Lineage Determination Report

T-Cell Receptor (TCR) Gene Rearrangement Analysis (Blood-derived cell lines only)

	TCR-αβ		TCR-γδ	
T Cell Clonality Assay:	☒Positive	☐Negative	☐Positive	☐Negative
Final Result:	☒T Cell Derived	☐Non-T Cell Derived	☐TBD	☐N/A

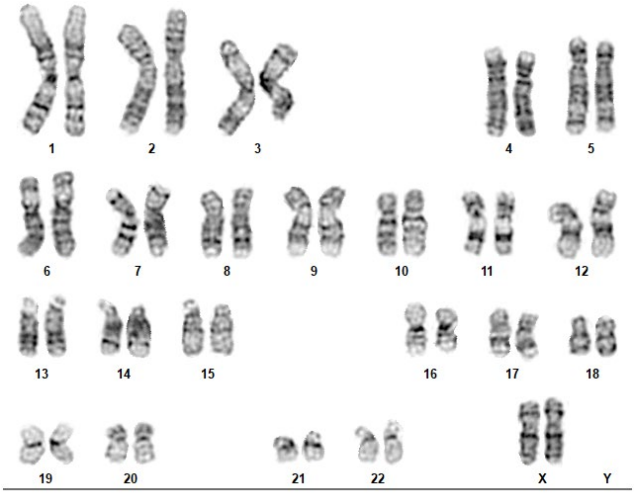
Assay Description:
Genomic DNA is extracted using the KingFisher Duo Prime Purification System (Thermo Scientific) and isolated using the MagMAX DNA Multi-Sample Ultra 2.0 Kit (Applied Biosystems), then resuspended to a final concentration of 100 µg/mL – 400 µg/mL in elution buffer. Using the TCRB + TCRG T-Cell Clonality Assay for Gel Detection (Invivoscribe), PCR is then carried out as per the manufacturer's protocol. The T-Cell Clonality Assay uses multiple consensus DNA primers which target conserved regions within the T-cell receptor β chain and γ chain genes, including the conserved framework, diversity, and joining regions. PCR products are analyzed using 6% Tris-borate-EDTA (TBE) gel electrophoresis with a 100bp ladder and gel red staining. Clonality is indicated as positive if any of the master mixes generate clonal band(s), and negative if no clonal band(s) are generated. For further details regarding clonal band product size, please refer to the TCRB + TCRG T-Cell Clonality Assay manual.

Reference:
Invivoscribe (2019) Instructions for Use TCRB + TCRG T-Cell Clonality Assay. Rev. G:3-15.

Chromosome Analysis Report

GTL-Banded Karyotype Analysis

Date Reported	2023-08-24
Sample	SCTi003-A Lot # 2307406009
Cell Line Sex	Female
Submitted Passage #	32
Date of Sample	2023-08-09
Specimen	Human iPSC
Results	46,XX



Cell	4
Slide	G02
Slide Type	Karyotype
Total Counted	20
Total Analyzed	8
Total Karyogrammed	4
Band Resolution	400 - 425

Interpretation:
This is a normal karyotype; no clonal abnormalities were detected 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 four 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”.

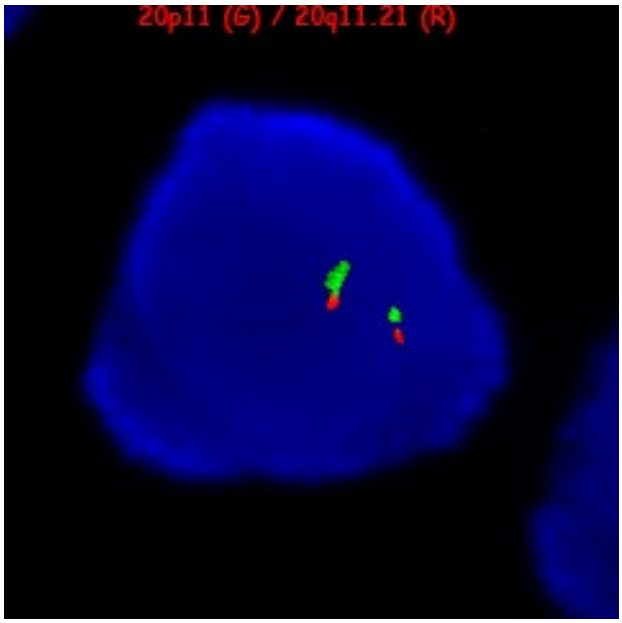
20q Status Report

Fluorescence In-Situ Hybridization (FISH) Analysis

Date Reported	2022-01-11
Sample	SCTi003-A Master Cell Bank
Cell Line Sex	Female
Submitted Passage #	26
Date of Sample	2021-11-30
Specimen	Human iPSC

Probe	# of cells with 2G1R pattern	# of cells with 1G1R pattern	# of cells with 2G2R pattern	# of cells with 2G3R pattern	# of cells with 1G2R pattern
20p11 (G) / BCL2L1 (R)	2 / 200 (1.0%)	1 / 200 (0.5%)	188 / 200 (94.0%)	8 / 200 (4.0%)	1 / 200 (0.5%)
Cutoff	4%	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 94.0% of two hundred interphase cells examined for the 20p11.21 and 20q11.21 (BCL2L1) regions.

Copy Number Variants (CNV) Report

Microarray Analysis

Date Reported	2023-09-15
Sample	SCTi003-A Lot # 2307406009
Cell Line Sex	Female
Submitted Passage #	32
Date of Sample	2023-08-09
Specimen	Human iPSC

Microarray Results arr[GRCh37] 7q34(142023396_142470490)x1,14q11.2(22327490_22784548)x1

Call Table:

Chr	Cytoband	Event (% Mosaic)	Estimated Copy Number	Start	End	Length (Base Pairs)	Gene Count
1	1p36.11	CN Loss	1	25,601,115	25,629,950	28,836	2
5	5p13.3	CN Gain	3	32,109,122	32,154,527	45,406	2
7	7p14.1	Homozygous Copy Loss	0	38,311,831	38,338,251	26,421	1
7	**7q34**	**CN Loss**	**1**	**142,023,396**	**142,470,490**	**447,095**	**2**
12	12p13.31	CN Gain	3	8,001,825	8,123,306	121,482	2
14	**14q11.2**	**CN Loss**	**1**	**22,327,490**	**22,784,548**	**457,059**	**0**
14	14q11.2	Homozygous Copy Loss	0	22,790,594	22,961,867	171,274	1
14	14q32.33	CN Loss	0-1	106,047,721	106,194,562	146,842	3
17	17q21.31	CN Gain	3	44,167,366	44,360,632	193,267	3

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

- A 0.447Mb loss on chromosome 7 was observed.
- A 0.457Mb loss on chromosome 14 was observed.

Copy Number Variants (CNV) Report (cont.)

Specifications:

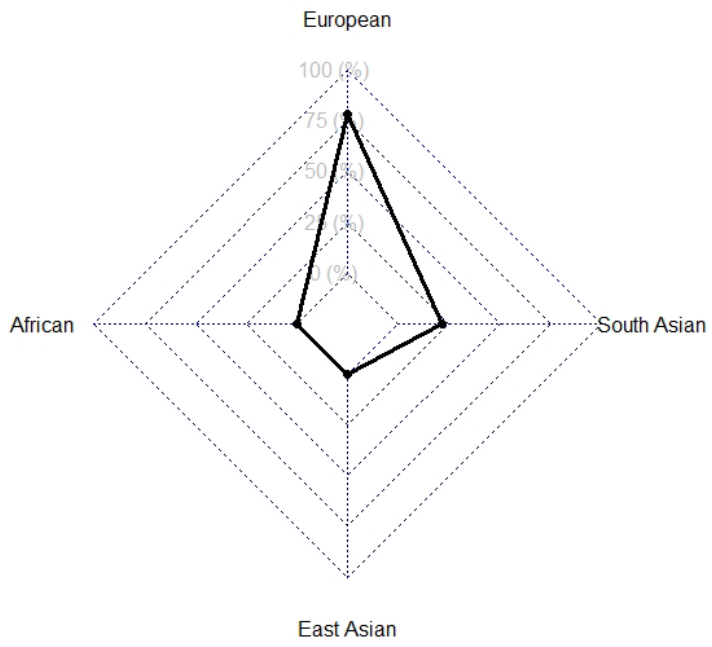
- Platform: Illumina: Global Diversity Array with Cytogenetics-8 (GDACyto)
- Marker coverage: 1,825,277 spanning whole human genome
- Analysis software: NxClinical (Via) 6.1 Software
- Array design, genomic position, genes and chromosome banding are based on genome build GRCh37/hg19.
- Aberrant copy 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.

Ancestry Report

Sample	SCTi003-A Master Cell Bank
Whole Exome Sequencing Report Date	2022-06-01
Ancestry Analysis Date	2022-06-23

EthSEQ Reference Database	**Ancestry:** European **Type:** Closest **Contribution:** European: 78.24% South Asian: 21.76%	

Assay Description:
DNA is purified from the sample and exon capture is performed using the SureSelect Human All Exon V6 (Agilent Technologies) for coding regions and splice junction sites of 20,000 human genes, covering 60 Mb of DNA. Post-capture libraries are sequenced using the NovaSeq 6000 System (Illumina) to a coverage of 50x using paired-end 150 nucleotide reads. Single Nucleotide Variants (SNVs) and insertions/deletions (indels) are detected across the entire exome after alignment to the GRCh38 human reference genome. Ancestry is calculated using the EthSEQ R Package and the reference model, described by Romanel (2017). The reference model is generated based on genotype data that encompasses 123,024 loci from individuals with known ancestries, grouped into four major populations: African, European, South Asian, and East Asian. A corresponding ancestry is reported if it falls inside the ancestral group set ("Inside"). The nearest ancestry is reported if it falls outside the ancestral group set ("Closest").

Reference:
Romanel, A. et al. (2017) EthSEQ: ethnicity annotation from whole exome sequencing data. Bioinformatics. 33(15):2402-04.

Genetic Variants Report

Sample	SCTi003-A Master Cell Bank
Whole Exome Sequencing Report Date	2022-06-01
ClinVar Analysis Date	2022-06-23

Results:

ClinVar Significance	Count
Pathogenic	3
Likely Pathogenic	1
Pathogenic or Likely Pathogenic	1
Risk Factor	17
Conflicting Interpretations of Pathogenicity	78
Uncertain Significance	61

Pathogenic or Likely Pathogenic Variants:

Gene	ClinVar ID	Molecular Consequence	Nucleotide Change	Protein Change
AGXT	204164	Splice acceptor	A>G	-
KLKB1	12033	Nonsense	C>T	R113*
SLC12A3	319889	Missense	C>T	R83W
NQO1	16809	Missense	G>A	P187S
RUNX1	988867	Missense	T>C	S397A

Assay Description:
DNA is purified from the sample and exon capture is performed using the SureSelect Human All Exon V6 (Agilent Technologies) for coding regions and splice junction sites of 20,000 human genes, covering 60 Mb of DNA. Post-capture libraries are sequenced using the NovaSeq 6000 System (Illumina) to a coverage of 50x using paired-end 150 nucleotide reads. Single Nucleotide Variants (SNVs) and insertions/deletions (indels) are detected across the entire exome after alignment to the GRCh38 human reference genome. Thresholding is performed to eliminate sequencing errors by only including regions covered by at least eight reads. In order to identify germline variants, heterozygous SNVs/indels are classified as regions in which minor alleles are represented by at least two unique reads and are present in at least 10% of all reads covering the locus. Resulting SNVs/indels are cross-referenced to ClinVar, a public archive of reports that detail relationships between human genetic variants and phenotypes (NCBI). Variants are classified based on the ClinVar significance score and pathogenic or likely pathogenic SNVs are reported.

TP53 and BCOR Status Report

Sample	SCTi003-A Master Cell Bank
Whole Exome Sequencing Report Date	2022-06-01
ClinVar Analysis Date	2022-06-23

Overview:

Gene	% Exon Covered by 8+ Reads	Average Exonic Read Depth	# Variants Detected
TP53	81.08%	42.83 (sd:55.35)	7
BCOR	73.89%	105.6 (sd:68.38)	5

TP53:

Locus	ClinVar ID	SNV	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chr17:7666380	-	A>C	-	Intronic	0	76	Germline homozygous
chr17:7674360	-	T>TT	-	Intronic insertion	12	12	Germline heterozygous
chr17:7674797	256603	T>C	-	Intronic	0	56	Germline homozygous
chr17:7675327	440348	C>T	-	Intronic	0	36	Germline homozygous
chr17:7675393	-	ΔTTT	-	Intronic deletion	1	10	Somatic
chr17:7676154	12351	G>C	P72R	Missense	0	62	Germline homozygous
chr17:7676483	137691	G>C	-	Intronic	2	55	Somatic

BCOR:

Locus	ClinVar ID	SNV	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chrX:40049780	-	C>T	-	3' UTR	16	15	Germline heterozygous
chrX:40052404	95772	C>A	-	Splice region	44	21	Germline heterozygous
chrX:40062607	674198	C>A	-	Intronic	10	24	Germline heterozygous
chrX:40073555	95767	G>A	-	Synonymous	19	65	Somatic
chrX:40074086	95764	A>G	-	Synonymous	94	109	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).

Assay Description:
DNA is purified from the sample and exon capture is performed using the SureSelect Human All Exon V6 (Agilent Technologies) for coding regions and splice junction sites of 20,000 human genes, covering 60 Mb of DNA. Post-capture libraries are sequenced using the NovaSeq 6000 System (Illumina) to a coverage of 50x using paired-end 150 nucleotide reads. Single Nucleotide Variants (SNVs) and insertions/deletions (indels) are detected across the entire exome after alignment to the GRCh38 human reference genome. Thresholding is performed to eliminate sequencing errors by only including regions covered by at least eight reads. In order to identify germline variants, heterozygous SNVs/indels are classified as regions in which minor alleles are represented by at least two unique reads and are present in at least 10% of all reads covering the locus. Resulting SNVs/indels are cross-referenced to ClinVar, a public archive of reports that detail relationships between human genetic variants and phenotypes (NCBI). All TP53 and BCOR variants meeting these specifications are reported. For TP53, variants are cross-referenced to those reported by Merkle et al. (2017).

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 Lot # 2307406009
Submitted Passage #	35
Analysis Date	2023-08-24
# of Events Analyzed	10,000

Results:

Marker	Expression
OCT4	96.8%
TRA-1-60	95.4%

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 Master Cell Bank
Submitted Passage #	30
Analysis Date	2022-03-11

Results:

Lineage	Marker	Expression
Endoderm	SOX17	85.5%
	CXCR4	95.6%
Mesoderm	BRACHYURY (T)	94.4%
	NCAM	91.6%
Ectoderm	PAX6	95.6%
	NESTIN	94.3%

Interpretation:
Following directed differentiation using the STEMdiff™ Trilineage Differentiation Kit (Cat # 05230), 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™ Trilineage Differentiation Kit (Cat # 05230). 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.