Characterization of Healthy Control Human iPSC Line, Male, SCTi004-A

Catalog #	200-0769
Lot #	2402408000

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Product Information

Product	Healthy Control Human iPSC Line, Male, SCTi004-A
Catalog #	200-0769
Lot #	2402408000
Format	~1 million viable cells per vial
Date Vialed	2024-02-29
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	SCTi004-A
Parent Material	SCTi004-A is a parent cell line
Cell Type	Human Induced Pluripotent Stem Cell (hiPSC)
Passage Number of Cell Banks*	**Master Cell Bank**: Passage 20 **Working Cell Bank**: Passage 23 **Commercial Cell Bank**: Passage 26 *This vial is from a SCTi004-A commercial cell bank and was cultured for 25 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); Non-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 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 ready for passaging at Day 7 - 8.

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

Donor Information

Age†	23	
Sex‡	Male	
Ethnicity and/or Race†	African American	
Ancestry‡	100.0% African 00.0% European	00.0% East Asian 00.0% South Asian
Diagnosis†	Clinically unaffected at donation	
Height‡	165 cm	
Weight‡	64.0 kg	
BMI‡	23.5 kg/m^2	
Blood Type‡	B+	
Tobacco Use†	Non-smoker	
HLA Haplotype‡	HLA Class **I:** A*34:02:01G, - B*44:03:02G, 52:01:02G C*07:01:01G, 16:01:01G	HLA Class **II:** DRB1*13:01:01G, 13:03:01G DRB3*01:01:02G, 02:02:01G DRB4*-, - DRB5*-, - DQB1*02:01:01G, 05:01:01G DPB1*01:01:01G, 04:02:01G

† Self-declared
‡ Calculated

Results Summary

Assessment	Analytical Method	Acceptance Criteria	Result
ViabilityCCB	Viability assessment performed on thawed cells using the NucleoCounter® NC-250™ by ChemoMetec	≥ 60% viable	Pass
RecoveryCCB	Cells recovered using specified thaw and culture recommendations	Recoverable attachment 24 hr. after plating and cells grow to confluency	Pass
Cell Line IdentityCCB	STR amplification performed using the Powerplex 16 HS System by Promega	Match	Pass
SterilityCCB	Presence or absence of bacterial and fungal organisms by incubation in TSB and FTB for 14 days	Negative	Pass
MycoplasmaCCB	Presence or absence of mycoplasma using the EZ-PCR™ Mycoplasma Detection Kit by Sartorius	Negative	Pass
Viral ScreenMCB	Human Comprehensive CLEAR PCR Panel	Negative	Pass
Parental Cell LineagePMB	Presence or absence of TCR Gene Rearrangements using the T cell clonality assay	No Specification	Reported
Residual VectorPMB	Genomic DNA analyzed by PCR	Negative	Pass
KaryotypeCCB	GTL Banding performed on 20 metaphase cells	Normal	Pass
20q AmplificationMCB	Fluorescence in situ hybridization (FISH)	Negative	Pass
Copy Number VariantsCCB	Genomic DNA analyzed using Illumina Global Diversity Array with Cytogenetics-8 (GDACyto)	No Specification	Reported
AncestryMCB	Whole exome sequencing data analyzed using EthSeq	No Specification	Reported
Genetic VariantsMCB	Whole exome sequencing data analyzed using ClinVar	No Specification	Reported
TP53 and BCOR StatusMCB	Whole exome sequencing data analyzed using ClinVar	No Specification	Reported
Undifferentiated StatusCCB	Three-passage assay and flow cytometry for undifferentiated cell markers	OCT4+, TRA-1-60+ ≥ 80%	Pass
PluripotencyMCB	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

CCBAssessment performed on the Commercial Cell Bank
WCBAssessment performed on the Working Cell Bank
MCBAssessment performed on the Master Cell Bank
PMBAssessment performed prior to the Master Cell Bank

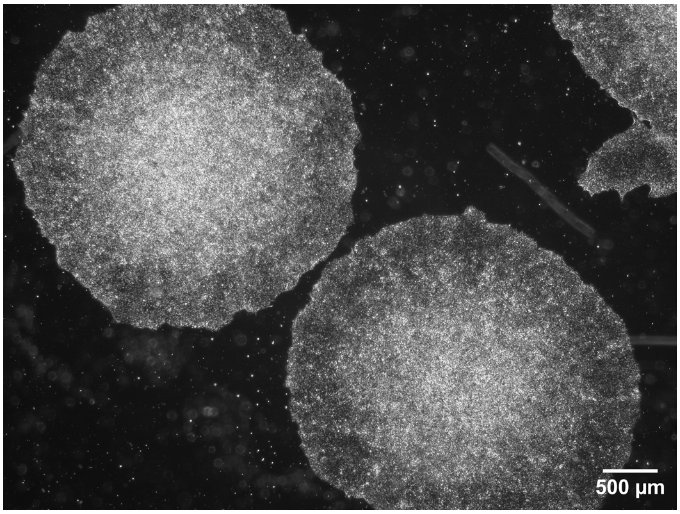
X VM 2024-05-15

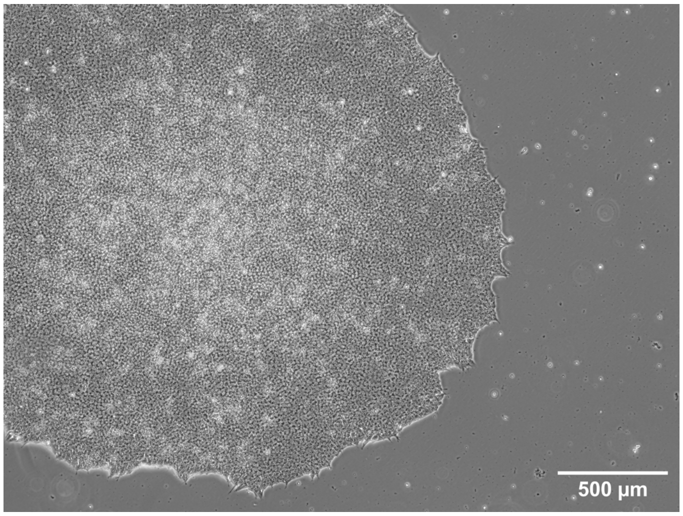
Approved by Initial & Date

Morphology Report

Sample	SCTi004-A Lot # 2402408000
Submitted Passage #	29
Analysis Date	2024-04-04

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	SCTi004-A Lot # 2402408000
Viability Platform	NucleoCounter® NC-250™
Viability Protocol	Viability and Cell Count - A100 and B Assay
Viability Analysis Date	2024-03-07
Recovery Completion Date	2024-03-14

Results:

Viability	65.4%
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

	SCTi004-A Lot # 2402408000	SCTi004-A Master Cell Bank
Samples Received Date	2024-04-02	2023-12-07
STR Amplification Date	2024-04-24	2024-01-03

Short Tandem Repeat (STR) Analysis

Sample Name	SCTi004-A Lot # 2402408000	SCTi004-A Master Cell Bank
CTR No. †	101510	99924
FGA	19, 24	19, 24
TPOX	11, 11	11, 11
D8S1179	14, 15	14, 15
vWA	14, 15	14, 15
Amelogenin	X, Y	X, Y
Penta_D	9, 11	9, 11
CSF1PO	9, 12	9, 12
D16S539	9, 11	9, 11
D7S820	11, 13	11, 13
D13S317	11, 12	11, 12
D5S818	8, 10	8, 10
Penta_E	8, 14	8, 14
D18S51	15, 15	15, 15
D21S11	28, 30	28, 30
TH01	8, 9	8, 9
D3S1358	15, 16	15, 16
Allelic Polymorphisms	28	28
Matches*	99924	101510
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 28 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	2024-03-15
Approval Date	2024-03-28

Diagnostic Summary

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

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

Bacteriology – Sterility Test – Broth Cultures: 2 Samples

	SCTi004-A Lot # 2402408000 Cryovial 1	SCTi004-A Lot # 2402408000 Cryovial 2	SCTi004-A Lot # 2402408000 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	2024-04-16
Assay Description	Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit (Sartorius).

Sample Name	Result	Interpretation
SCTi004-A **Lot # 2402408000**	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma
Positive (+) Control	Positive	
Negative (-) Control	Negative	

Viral Screen Report

Collection Date	2024-01-29
Approval Date	2024-02-02

Molecular Diagnostics – Infectious Disease PCR
Human Comprehensive CLEAR Panel

	SCTi004-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	-

	SCTi004-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

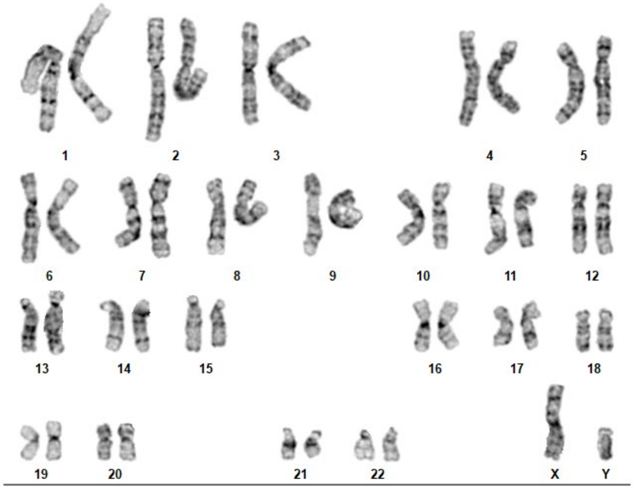
Assay Description:
Genomic DNA is extracted using the KingFisher Duo Prime Purification System (Thermo Scientific) and isolated using the MagMAX DNA Multi-Sample Ultra 2.0 Kit (Applied Biosystems), then resuspended to a final concentration of 100 µg/mL – 400 µg/mL in elution buffer. Using the TCRB + 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	2024-04-17
Sample	SCTi004-A Lot # 2402408000
Cell Line Sex	Male
Submitted Passage #	26
Date of Sample	2024-04-02
Specimen	Human iPSC
Results	46,XY



Cell	33
Slide	G01
Slide Type	Karyotype
Total Counted	20
Total Analyzed	8
Total Karyogrammed	4
Band Resolution	400 - 450

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”.

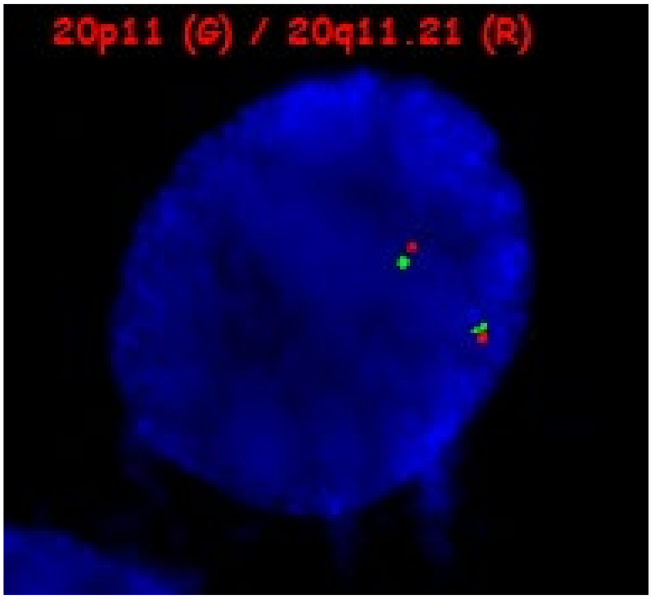
20q Status Report

Fluorescence In-Situ Hybridization (FISH) Analysis

Date Reported	2023-12-02
Sample	SCTi004-A Master Cell Bank
Cell Line Sex	Male
Submitted Passage #	20
Date of Sample	2023-11-15
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**	**# of cells with 3G2R pattern**
20p11 (G) / BCL2L1 (R)	4 / 200 (2.0%)	2 / 200 (1.0%)	184 / 200 (92.0%)	8 / 200 (4.0%)	1 / 200 (0.5%)	1 / 200 (0.5%)
Cutoff	4%	4%	N/A	5%	4%	3%

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



Interpretation:
There is no evidence for aneusomy of chromosome 20. Two probe signals were observed in 92.0% of two hundred interphase cells examined for the 20p11.21 and 20q11.21 (BCL2L1) regions.

Copy Number Variants (CNV) Report

Microarray Analysis

Date Reported	2024-05-03
Sample	SCTi004-A Lot # 2402408000
Cell Line Sex	Male
Submitted Passage #	26
Date of Sample	2024-04-02
Specimen	Human iPSC

Microarray Results arr(X,Y)x1,(1-22)x2

Call Table:

Chr	Cytoband	Event (% Mosaic)	Estimated Copy Number	Start	End	Length (Base Pairs)	Gene Count
1	1q31.3	CN Loss	1	196,763,107	196,887,425	124,319	3
3	3p24.2	CN Loss	1	24,766,655	24,801,709	35,055	0
5	5p15.33	CN Gain	3	678,046	821,463	143,418	3
11	11q25	CN Loss	1	134,152,030	134,212,747	60,718	2
17	17q12	CN Gain	4	34,437,963	34,664,830	226,868	5

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

Copy Number Variants (CNV) Report (cont.)

Specifications:

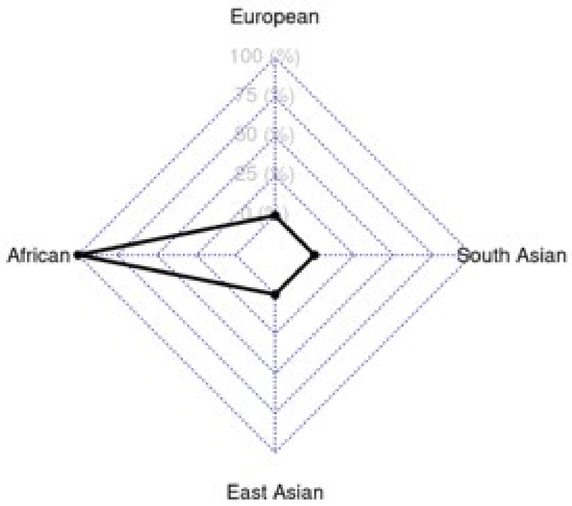
- Platform: Illumina: Global Diversity Array with Cytogenetics-8 (GDACyto)
- Marker coverage: 1,825,277 spanning whole human genome
- Analysis software: NxClinical (Via) 6.1 Software
- Array design, genomic position, genes and chromosome banding are based on genome build GRCh37/hg19.
- Aberrant copy 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	SCTi004-A Master Cell Bank
Whole Exome Sequencing Report Date	2024-02-08

EthSEQ Reference Database	**Ancestry:** African **Type:** Closest **Contribution:** African: 100.00%	

Analysis 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. Data are analyzed following GATK4 best practices. Briefly, raw reads are aligned to the reference genome GRCh38. Alignment refinements are applied to improve the quality of the alignment and to generate analysis-ready reads. Variant calling for SNPs and indels is performed using the mapped reads. The variant list is filtered to retain high-confidence variants. 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	SCTi004-A Master Cell Bank
Whole Exome Sequencing Report Date	2024-02-08
ClinVar Version Date	2024-02-15

Pathogenic or Likely Pathogenic Variants:

Gene	ClinVar ID	Coordinates	Molecular Consequence	Nucleotide Change	Protein Change
COL4A	191312	chr2:227028004	Missense variant	c.3979G>A	V1327M
DCAF17	191260	chr2:171448667	Intron variant	c.322-14C>T	-
ENPP1	13589	chr6:131851228	Missense variant	c.517A>C	K173Q
HBB	15333	chr11:5227002	Missense variant	c.20A>T	E7V
ITPKB	1705897	chr1:226735804	Missense variant	c.1655C>A	P552Q
ITPKB	1705899	chr1:226736237	Missense variant	c.1222T>G	S408A
ITPKB	1705896	chr1:226737174	Disruptive inframe deletion	c.267CAGCGGCAG[1]	p.91GSS[1]
KCNE2	6053	chr21:34370639	Missense variant	c.161T>C	M54T
LDLRAP1	4776	chr1:25563142	Missense variant	c.605C>A	S202Y
MEFV	2542	chr16:3254626	Missense variant	c.442G>C	E148Q
PDGFRA	133374	chr4:54281602	Intron variant	c.2323+1120C>T	T782M
SLC3A1	336214	chr2:44320435	Missense variant	c.1854G>A	M618I
SLC9B1	2503084	chr4:102901325	Frameshift variant	c.1338_1339del	L447fs
TUBB2B	381699	chr6:3225346	Missense variant	c.743C>T	A248V
UGT1A	12288	chr2:233757013	Upstream gene variant	c.862-10021T>G	-
VDR	308887	chr12:47879112	Start lost	c.2T>C	M1T

Genetic Variants Report (cont.)

Analysis 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. Data are analyzed following GATK4 best practices. Briefly, raw reads are aligned to the reference genome GRCh38. Alignment refinements are applied to improve the quality of the alignment and to generate analysis-ready reads. Variant calling for SNPs and indels is performed using the mapped reads. The variant list is filtered to retain high-confidence variants. Variants are annotated with predicted functional annotations and ClinVar IDs. Identified variants are assigned a score by CNNScoreVariants, which uses a Convolutional Neural Network to score each variant with the model's quality prediction. The model incorporates the reference sequence surrounding the variant and the variant features. A set of resources is used to determine 'true sites' of genomic variation (HapMap 3.3, Omni 2.5M SNP chip array, and Mills and 1000G Gold Standard Indels). Following GATK Best Practices, variants with a CNN score higher than the 99.95th percentile of SNPs in the resources, and higher than the 99.4th percentile for indels are retained.

ClinVar entries from either WES or WGS require 2 or more supporting ALT reads and an assertion criterion to be considered (one gold star or more in ClinVar). Variants are classified as pathogenic in cases where the reported status is Pathogenic or Likely Pathogenic in the ClinVar database.

Note that the classification of variants as pathogenic or likely pathogenic is provided by ClinVar and STEMCELL has not verified its accuracy. Further, this reflects knowledge at the time the report was generated, and the classification of variants as pathogenic or likely pathogenic may change over time.

TP53 and BCOR Status Report

Sample	SCTi004-A Master Cell Bank
Whole Exome Sequencing Report Date	2024-02-08
ClinVar Version Date	2024-02-15

Overview:

Gene	% Exon Covered by 15+ Reads	Average Exonic Read Depth	# Exonic or Impact Variants Detected
TP53	100.00%	50.47 (sd:10.01)	1
BCOR	91.41%	23.59 (sd:6.96)	1

TP53:

Locus	ClinVar ID	Nucleotide Change	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chr17:7688850	261001	c.202C>G	R68G	Missense variant	0	50	Germline homozygous

BCOR:

Locus	ClinVar ID	Nucleotide Change	Protein Change	Variant Type	# Ref Reads	# Alt Reads	Inferred Inheritance
chrX:40074086	95764	c.1260T>C	D420D	Synonymous variant	0	37	Germline homozygous

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:

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. Data are analyzed following GATK4 best practices. Briefly, raw reads are aligned to the reference genome GRCh38. Alignment refinements are applied to improve the quality of the alignment and to generate analysis-ready reads. Variant calling for SNPs and indels is performed using the mapped reads. The variant list is filtered to retain high-confidence variants. Variants are annotated with predicted functional annotations and ClinVar IDs. Identified variants are assigned a score by CNNScoreVariants, which uses a Convolutional Neural Network to score each variant with the model's quality prediction. The model incorporates the reference sequence surrounding the variant and the variant features. A set of resources is used to determine 'true sites' of genomic variation (HapMap 3.3, Omni 2.5M SNP chip array, and Mills and 1000G Gold Standard Indels). Following GATK Best Practices, variants with a CNN score higher than the 99.95th percentile of SNPs in the resources, and higher than the 99.4th percentile for indels are retained.

Exonic TP53 and BCOR variants or those predicted to have an impact on TP53 or BCOR meeting these specifications and with 2 supporting ALT reads 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	SCTi004-A Lot # 2402408000
Submitted Passage #	29
Analysis Date	2024-04-04
# of Events Analyzed	10,000

Results:

Marker	Expression
OCT4	98.0%
TRA-1-60	99.6%

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	SCTi004-A Master Cell Bank
Submitted Passage #	22
Analysis Date	2024-01-24

Results:

Lineage	Marker	Expression
Endoderm	SOX17	98.2%
	CXCR4	96.5%
Mesoderm	BRACHYURY (T)	94.8%
	NCAM	99.0%
Ectoderm	PAX6	98.5%
	NESTIN	96.0%

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.