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

Catalog # 200-0511
Lot # 2411401001

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# Product Information

| | |
|---|---|
| **Product** | Healthy Control Human iPSC Line, Female, SCTi003-A |
| **Catalog #** | 200-0511 |
| **Lot #** | 2411401001 |
| **Format** | ~1 million viable cells per vial |
| **Date Vialed** | 2024-11-18 |
| **Country of Manufacture** | US |
| **Stability, Storage, and Use Information** | Product stable at -135°C or colder for 12 months from date of receipt. Thawed samples must be used immediately.<br><br>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.<br><br>FOR IN VITRO RESEARCH USE ONLY. NOT APPROVED FOR DIAGNOSTIC, THERAPEUTIC, OR CLINICAL APPLICATIONS.<br><br>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<br>**Working Cell Bank**: Passage 29<br>**Commercial Cell Bank**: Passage 32<br><br>*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 6 - 7. |

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<br>100% European | 0% East Asian<br>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:<br><br>A*24:02:01G, 26:01:01G<br>B*07:02:01G, -<br>C*07:02:01G, -<br>E*01:01:01G, -<br>F*01:01:01G, 01:04<br>G*01:01:02G, 01:04:01G | HLA Class II:<br><br>DRB1*15:01:01G, -<br>DRB3*-, -<br>DRB4*-, -<br>DQA1*01:02:01G, -<br>DQB1*06:02:01G, -<br>DPA1*01:03:01G, -<br>DPB1*02:01:02G, 04:01:01G |

† Self-declared
‡ Calculated

## 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 and whole genome sequencing data analyzed using EthSeq | No Specification | Reported |
| **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 using the STEMdiff™ Trilineage Differentiation Kit | **Endoderm:** CXCR4+, SOX17+ ≥ 70%<br>**Mesoderm:** T+, NCAM+ ≥ 70%<br>**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

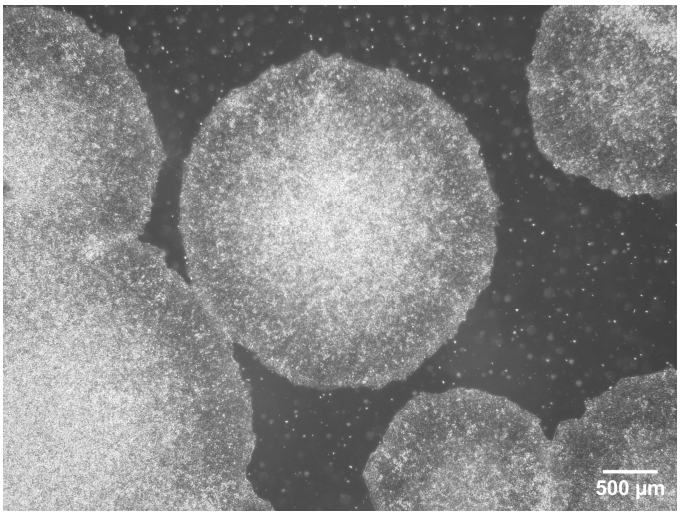
X vm 2025-06-18

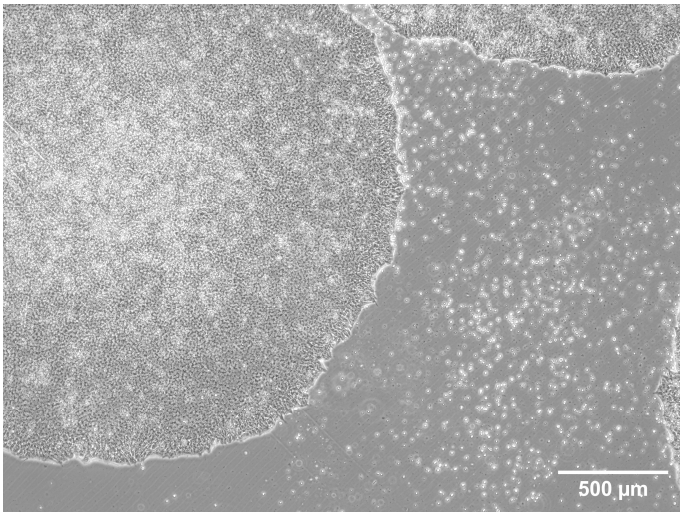
Approved by Initial & Date

# Morphology Report

| | |
|---|---|
| **Sample** | SCTi003-A Lot # 2411401001 |
| **Submitted Passage #** | 35 |
| **Analysis Date** | 2025-02-05 |

**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 # 2411401001 |
| **Viability Platform** | NucleoCounter® NC-250™ |
| **Viability Protocol** | Viability and Cell Count - A100 and B Assay |
| **Viability Analysis Date** | 2025-01-08 |
| **Recovery Completion Date** | 2025-01-15 |

**Results:**

| | |
|---|---|
| **Viability** | 83.3% |
| **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 # 2411401001 | SCTi003-A Master Cell Bank |
|---|---|---|
| **Samples Received Date** | 2025-01-23 | 2021-11-30 |
| **STR Amplification Date** | 2025-02-03 | 2021-12-20 |

## Short Tandem Repeat (STR) Analysis

| Sample Name | SCTi003-A Lot # 2411401001 | SCTi003-A Master Cell Bank |
|---|---|---|
| **CTR No.** † | 105523 | 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 | 105523 |
| **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** | 2025-01-22 |
| **Approval Date** | 2025-02-06 |

## Diagnostic Summary

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

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

## Bacteriology – Sterility Test – Broth Cultures: 2 Samples

| | **SCTi003-A Lot # 2411401001 Cryovial 1** | **SCTi003-A Lot # 2411401001 Cryovial 2** |
|---|---|---|
| ***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** | 2025-02-03 |
| **Assay Description** | Sample is tested for presence of mycoplasma using EZ-PCR™ Mycoplasma Detection Kit (Sartorius). |

| Sample Name | Result | Interpretation |
|---|---|---|
| **SCTi003-A<br>Lot # 2411401001** | 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 |

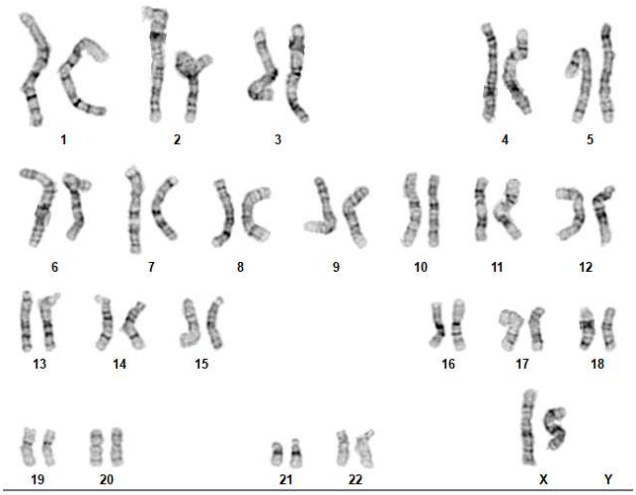
**Assay Description:**
Genomic DNA is extracted using the KingFisher Duo Prime Purification System (Thermo Scientific) and isolated using the MagMAX DNA Multi-Sample Ultra 2.0 Kit (Applied Biosystems), then resuspended to a final concentration of 100 µg/mL – 400 µg/mL in elution buffer. Using the TCRB + 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** | 2025-02-24 |
| **Sample** | SCTi003-A Lot # 2411401001 |
| **Cell Line Sex** | Female |
| **Submitted Passage #** | 32 |
| **Date of Sample** | 2025-02-11 |
| **Specimen** | Human iPSC |
| **Results** | 46,XX |




| | |
|---|---|
| **Cell** | 60 |
| **Slide** | G02 |
| **Slide Type** | Karyotype |
| **Total Counted** | 20 |
| **Total Analyzed** | 9 |
| **Total Karyogrammed** | 4 |
| **Band Resolution** | 450 - 475 |

**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”.

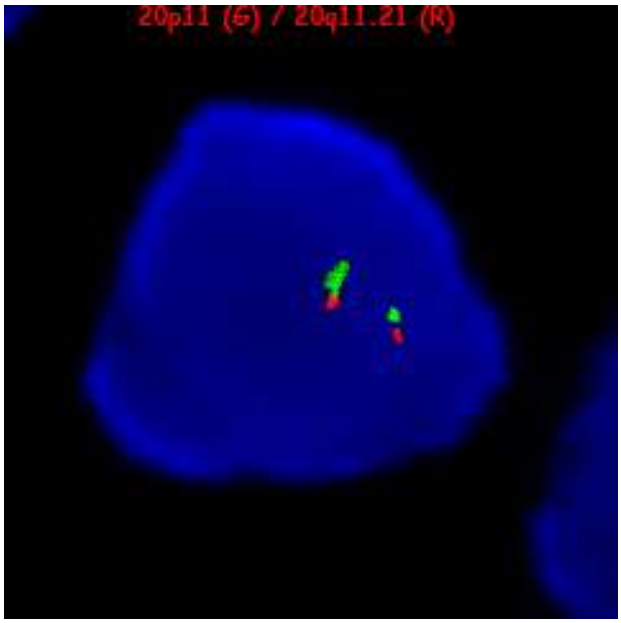
# 20q Status Report

## 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** | 2025-03-02 |
| **Sample** | SCTi003-A Lot # 2411401001 |
| **Cell Line Sex** | Female |
| **Submitted Passage #** | 32 |
| **Date of Sample** | 2025-01-23 |
| **Specimen** | Human iPSC |

Microarray Results arr[GRCh37] 7q34(142020925_142495156)x1,14q11.2(22321109_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** | **142,020,925** | **142,495,156** | **474,232** | **2** |
| **14** | **14q11.2** | **Loss** | **1** | **22,321,109** | **22,789,110** | **468,002** | **0** |
| 14 | 14q11.2 | Homozygous Loss | 0 | 22,790,594 | 22,961,867 | 171,274 | 1 |
| 14 | 14q32.33 | Loss | 0-1 | 106,047,905 | 106,236,278 | 188,374 | 3 |

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

- A 0.47Mb loss on chromosome 7 was observed.
- A 0.47Mb loss on chromosome 14 was observed.

# Copy Number Variants (CNV) Report (cont.)

**Specifications:**

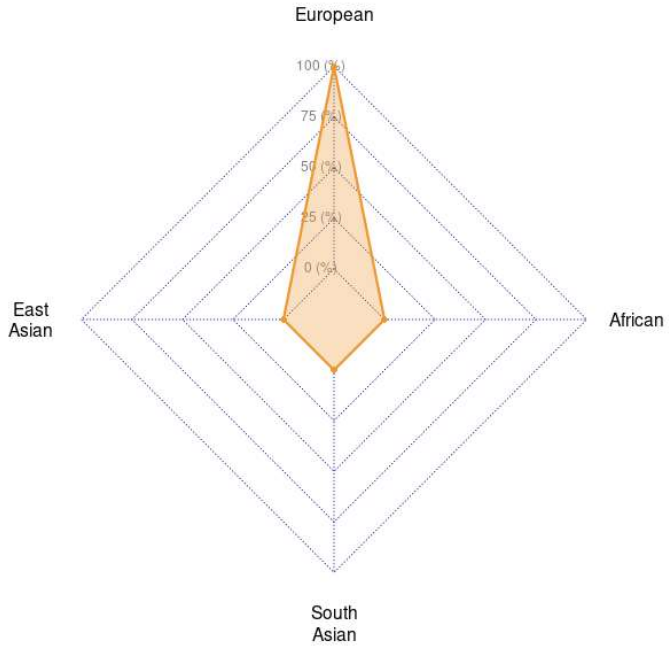
- Platform: Illumina: Global Diversity Array with Cytogenetics-8 (GDACyto)
- Marker coverage: 1,825,277 spanning whole human genome
- Analysis software: Bionano: Via™ Software
- Array design, genomic position, genes and chromosome banding are based on genome build GRCh37/hg19.
- Aberrant copy number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

# Ancestry Report

| | |
|---|---|
| **Sample** | SCTi003-A Master Cell Bank |
| **Whole Exome and Whole Genome Sequencing Report Date** | 2024-10-18 |

| | | |
|---|---|---|
| **EthSEQ Reference Database** | **Ancestry:** European<br><br>**Type:** Inside<br><br>**Contribution:**<br>European: 100% |  |

**Analysis Description:**
Genomic DNA from the sample was randomly fragmented into shorter pieces. These fragments were end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified. The libraries were then sequenced using the NovaSeq 6000 system (Illumina) with paired-end 150 nucleotide reads, achieving a coverage of 50x. To enhance coverage of coding regions, exonic data was added through exon capture using the SureSelect Human All Exon V6 kit (Agilent Technologies). This process targeted the coding regions and splice junction sites of 20,000 human genes, covering approximately 60 Mb of DNA. Global single nucleotide variants (SNVs) and insertions/deletions (indels) were identified by aligning the sequencing reads to the GRCh38 human reference genome following GATK best practices (v4.6.1.0).

Ancestry was calculated using the EthSEQ R package (v3.0.2) and a reference model described by Romanel et al. (2017). The reference model included genotype data from 233,887 loci representing individuals with known ancestries, categorized into four major populations: African, European, South Asian, and East Asian. If a sample's ancestry fell within one of these ancestral groups, it was reported as "Inside." If the sample's ancestry lay outside the predefined groups, the nearest ancestry was reported as "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 and Whole Genome Sequencing Report Date** | 2024-10-18 |
| **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 | C130R |
| 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 |
| PRSS1,TRB | 38363 | chr7:142750561 | Missense variant | c.47C>T | A16V |
| 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:**
Genomic DNA from the sample was randomly fragmented into shorter pieces. These fragments were end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified. The libraries were then sequenced using the NovaSeq 6000 System (Illumina) with paired-end 150 nucleotide reads, achieving a coverage of 50x. To enhance coverage of coding regions, exonic data was added through exon capture using the SureSelect Human All Exon V6 kit (Agilent Technologies). This process targeted the coding regions and splice junction sites of 20,000 human genes, covering approximately 60 Mb of DNA. Global single nucleotide variants (SNVs) and insertions/deletions (indels) were identified by aligning the sequencing reads to the GRCh38 human reference genome following GATK best practices (v4.6.1.0).

SNVs and indels were retained if they met the CNN filtering threshold and were supported by at least two reads. The resulting variants were cross-referenced with ClinVar (v2024-02), a publicly accessible database that links human genetic variants to associated phenotypes (NCBI). Variants were then classified based on the ClinVar significance score, with only those identified as pathogenic or likely pathogenic reported.

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** | SCTi003-A Master Cell Bank |
| **Whole Exome and Whole Genome Sequencing Report Date** | 2024-10-18 |
| **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% | 109.63 (sd:61.78) | 1 |
| BCOR | 99.60% | 101.90 (sd:76.73) | 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 | 162 | 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 | 63 | 76 | Germline heterozygous |
| chrX:40074086 | 95764 | c.1260T>C | p.Asp420Asp | Synonymous variant | 127 | 134 | 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:**
Genomic DNA from the sample was randomly fragmented into shorter pieces. These fragments were end-repaired, A-tailed, and ligated with Illumina adapters. The adapter-ligated fragments were size-selected, PCR-amplified, and purified. The libraries were then sequenced using the NovaSeq 6000 (Illumina) with paired-end 150 nucleotide reads, achieving a coverage of 50x. To enhance coverage of coding regions, exonic data was added through exon capture using the SureSelect Human All Exon V6 kit (Agilent Technologies). This process targeted the coding regions and splice junction sites of 20,000 human genes, covering approximately 60 Mb of DNA. Global single nucleotide variants (SNVs) and insertions/deletions (indels) were identified by aligning the sequencing reads to the GRCh38 human reference genome following GATK best practices (v4.6.1.0).

SNVs and indels were retained if they met the CNN filtering threshold and were supported by at least two reads. The resulting variants were cross-referenced with ClinVar (v2024-02), a publicly accessible database that links human genetic variants to associated phenotypes (NCBI). 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).

**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 # 2411401001 |
| **Submitted Passage #** | 35 |
| **Analysis Date** | 2025-02-05 |
| **# of Events Analyzed** | 10,000 |

**Results:**

| Marker | Expression |
|---|---|
| OCT4 | 81.3% |
| TRA-1-60 | 97.1% |

**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.