Certificate of Analysis

Thaw and Culture Details

Cell Line Name	**STAN159i-336C3**	
WiCell Lot Number	**DB44543**	
Provider/Client	Stanford University – Laboratory of Dr. Thomas Quetermous	
Banked By	Icahn School of Medicine at Mount Sinai Stem Cell Core	
Thaw and Culture Recommendations	WiCell recommends thawing 1 vial into 2 wells of a 6 well plate using mTeSR™Plus and Matrigel®. WiCell recommends passaging with ROCK Inhibitor.	
Protocol	WiCell Feeder Independent Pluripotent Stem Cell Protocol	
Culture Platform Prior to Freeze	Medium: mTeSR™1	Matrix: Matrigel®
Passage Number	p12 Cells were cultured for 12 passages prior to freeze and post colony selection. Plated cells at thaw should be labeled passage 13.	
Date Vialed	19-August-2015	
Vial Label	ISMMS 336i C3P12 AP 081915	
Biosafety and Use Information	Appropriate biosafety precautions should be followed when working with these cells. The end user is responsible for ensuring that the cells are handled and stored in an appropriate manner. WiCell is not responsible for damages or injuries that may result from the use of these cells. Cells distributed by WiCell are intended for research purposes only and are not intended for use in humans.	

Certificate of Analysis

Results

Test Description	Test Provider	Test Method	Test Specification	Result
Karyotype	WiCell	G-T-L Banding performed on 20 metaphase cells	Expected karyotype	See Report
	Results: 46,XX,i(20)(q10)[3]/47,XX,+i(12)(p10)[2]/46,XX[14] Nonclonal Findings: 47,XX,+20 ***Interpretation:*** This is an abnormal karyotype. There are two unrelated abnormal clones. The cells in the main clone (three of twenty cells examined; representative image on the left) contain an isochromosome of the long (q) arm of chromosome 20. This imbalance results in trisomy for 20q and monosomy for 20p. Gain of chromosome 20q is a recurrent acquired abnormality in pluripotent stem cell cultures. The cells in the second clone (two of twenty cells examined; representative image on the right) contain an additional isochromosome of the short (p) arm of chromosome 12. This imbalance results in tetrasomy for 12p. Gain of chromosome 12p is a recurrent acquired abnormality in pluripotent stem cell cultures There is a nonclonal finding, listed above, which contains a chromosomal aberration (gain of chromosome 20) recurrently acquired in pluripotent stem cell cultures. Nonclonal findings may result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism. No other clonal abnormalities were detected at the stated band level of resolution.		
Post-Thaw Viable Cell Recovery	WiCell	Thaw using specified Thaw & Culture Recommendations	Recoverable attachment after passage	Pass
Identity by STR	WiCell	PowerPlex 16 HS System by Promega™	Defines STR profile of deposited cell line	See Report
Mycoplasma	WiCell	PCR	Amplification of mycoplasma specific DNA detected with negative result	Pass
Sterility	Steris	Native Product Direct Transfer using FTM and TSB (ST/07)	Negative for growth following 14 days of culture	Pass

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Testing Reported by Provider

Test Description	Method	Result
Mycoplasma	Lonza MycoAlert kit	Negative

The Provider stated that some or all of the additional analyses listed below may have been performed for this cell line. For more information, publication and dbGaP links, where available, are provided on the cell line specific web page on the WiCell website.

- RNA-Seq
- Whole Genome Sequencing
- Infinium® Expanded Multi-Ethnic Genotyping Array (MEGA^{EX})

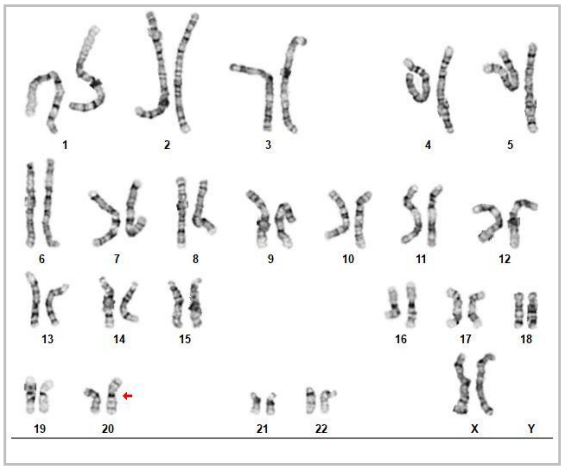
Approval Date	WiCell Quality Assurance Approval
25-January-2023	1/25/2023 X HEB HEB WiCell Quality Assurance Signed by: Bruner, Haley

Chromosome Analysis Report: 095077

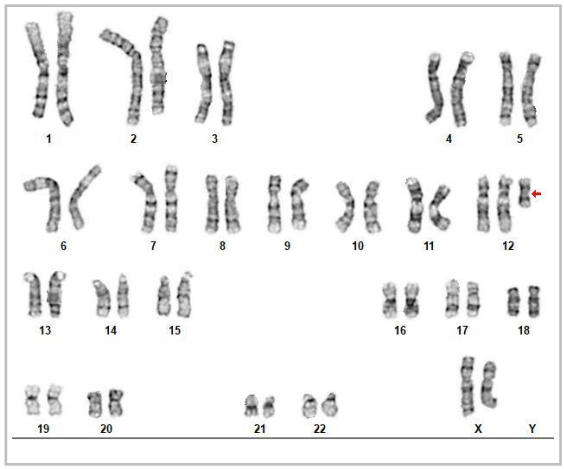
Date Reported: Monday, December 26, 2022
Cell Line Sex: Female
Cell Line: STAN159i-336C3-DB44543
Reason for Testing: LOT_RELEASE
Submitted Passage #: 14
Date of Sample: 12/7/2022
Investigator: WiCell Stem Cell Bank, WiCell
Specimen: Human IPSC
Results: 46,XX,i(20)(q10)[3]/47,XX,+i(12)(p10)[2]/46,XX[14]

Nonclonal Findings: 47,XX,+20

Cell: 20 **Slide: G02** **Slide Type: Karyotyping**



Cell: 47 **Slide: G01** **Slide Type: Karyotyping**



Total Counted: 20
Total Analyzed: 9
Total Karyogrammed: 4
Band Resolution: 400 - 500

Interpretation:

This is an abnormal karyotype. There are two unrelated abnormal clones.

The cells in the main clone (three of twenty cells examined; representative image on the left) contain an isochromosome of the long (q) arm of chromosome 20. This imbalance results in trisomy for 20q and monosomy for 20p. Gain of chromosome 20q is a recurrent acquired abnormality in pluripotent stem cell cultures.

The cells in the second clone (two of twenty cells examined; representative image on the right) contain an additional isochromosome of the short (p) arm of chromosome 12. This imbalance results in tetrasomy for 12p. Gain of chromosome 12p is a recurrent acquired abnormality in pluripotent stem cell cultures

There is a nonclonal finding, listed above, which contains a chromosomal aberration (gain of chromosome 20) recurrently acquired in pluripotent stem cell cultures. Nonclonal findings may result from technical artifact, but may be due to a developing clonal abnormality or to low-level mosaicism.

No other clonal abnormalities were detected at the stated band level of resolution.

Completed by: Dawn Davis, CG(ASCP)

Reviewed and Interpreted by: ***Xiangqiang Shao, PhD***

For internal use only

Date:_____ Sent By:____ Sent To:_____ QC Review By: ____

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".

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 19Dec22
STR Amplification Date: 04Jan23

Sample Name	STAN159i-336C3-DB44543 p13
WiCell CTR No.[1]	95230
FGA	Identifying information has been redacted to protect donor confidentiality. If more information is required, please contact info@wicell.org
TPOX	
D8S1179	
vWA	
Amelogenin	
Penta_D	
CSF1PO	
D16S539	
D7S820	
D13S317	
D5S818	
Penta_E	
D18S51	
D21S11	
TH01	
D3S1358	
Allelic Polymorphisms	28
Matches*	95023
Comments	

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

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

Short Tandem Repeat

Requestor: WiCell Stem Cell Bank, WiCell
Samples Received: 19Dec22
STR Amplification Date: 04Jan23

Assay Description: STR analysis is performed using the PowerPlex 16 HS System by Promega™. Results are reported as 13 CODIS STR markers, Amelogenin for gender 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 suggests 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%.

1/5/2023 X Justin Hobson Tech #1 Characterization Signed by: Hobson, Justin	1/6/2023 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	1/6/2023 X Dawn Graham QA Review Quality Assurance Signed by: Graham, Dawn

Mycoplasma Assay Report

PCR-based assay performed by WiCell

WiCell Stem Cell Bank, WiCell

20Dec22

Sample Name	Result	Interpretation
STAN159i-336C3-DB44543 p13 (95230)	Negative	Band was not seen at 270bp, indicating the absence of mycoplasma.
Positive (+) Control	Positive	
Negative (-) Control	Negative	

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

12/22/2022 X Kaylie Petersen Tech #1 Characterization Signed by: Petersen, Kaylie	12/22/2022 X Amber Kuhn Tech #2 Characterization Signed by: Kuhn, Amber	12/22/2022 X Dawn Graham QA Review Quality Assurance Signed by: Graham, Dawn

A gel image is available upon request.

Native Product Sterility Report

STERIS

CORRECTED REPORT #2

WiCell
504 S Rosa Road, Rm 101
Madison, WI 53719

SAMPLE #: 22031046
DATE RECEIVED: 17-Mar-22
TEST INITIATED: 28-Mar-22
TEST COMPLETED: 11-Apr-22

SAMPLE NAME / DESCRIPTION:
WA09-WB67843
WA09-WB67844
UCSD241i-APP2-3-WB67845
iPS(IMR90)-4-WB67846
iPS(IMR90)-4-WB67847
STAN158i-336C2-DB44540
STAN159i-336C3-DB44543
STAN122i-193C1-DB35800
STAN162i-345C2-DB38177
STAN121i-193C2-DB35803

UNIQUE IDENTIFIER: N/A

TEST RESULTS:

# Tested	# Positives (Growth)	- Control
10	4	2 Negatives

TEST SUMMARY:

# Samples	Media Type	Volume (mL)	Incubation Temperature (° C)	Incubation Duration (Days)
10	TSB	40	20-25	14
10	FTG	10	30-35	14

REFERENCE: Processed according to LAB-003: Sterility Test Procedure
PD #: 000053
TEST METHODOLOGY: USP - Direct Transfer

COMMENTS: Corrected report revised due to updated comments.

Report revised due to updated Sample Name/Description.

Sample labeled STAN122i-193C1-DB35800 positive for TSB and FTG
Sample labeled STAN121i-193C2-DB35803 positive for TSB and FTG

REVIEWED BY _____ DATE 18/APR2022